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SOME MECHANISMS UNDERLYING THE ELECTRICAL
AND MECHANICAL ACTIVITY OF THE DOG
SMALL INTESTINE

by



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↓
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A THESIS
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FACULTY OF GRADUATE STUDIES

The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies for acceptance, a thesis entitled "Some Mechanisms Underlying the Electrical and Mechanical Activity of the Dog Small Intestine" submitted by Khin Kyi Kyi in partial fulfilment of the requirements for the degree of Doctor of Philosophy.

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ABSTRACT

Spontaneous electrical activity of the dog small intestine in vivo consists of rhythmic slow potentials from periodic depolarisation of the longitudinal muscle cells. These may enlarge and generate fast spikes during contractions. Intra-arterial and intravenous epinephrine (E), norepinephrine (NE) and isopropyl norepinephrine (INE) in an active segment produced relaxation and disappearance of contractions with concomitant disappearance of spikes. Changes in frequency and amplitude of the slow waves occurred with larger doses. Order of potency of catecholamines in the controls was $E > NE \gg INE$ suggesting predominance of alpha-receptors. The order of potency changed to $INE > E > NE$ after intravenous administration of tolazoline indicating the presence of beta-receptors. Each drug appeared to act on both receptors, the effects being predominant on one receptor. Inhibitory effects of catecholamines in the intestine were not secondary to vasoconstriction produced by the drugs. Cocaine did not potentiate the effects of intra-arterial perfusions of NE. It seemed to do so when the catecholamine was given intravenously. The absence of cocaine potentiation of intra-arterial NE may result from the absence of adrenergic nerve endings in the vicinity of the intestinal muscle or from inefficient uptake mechanism in the intestine.

The effects of prolonged ischaemia on the electrical and mechanical activity and on nerve responses to transmural stimulation of the jejunum were studied in acute and chronic preparations in dogs. Distorted, irregular slow waves with low frequency and low conduction velocity were recorded in vivo in post-ischaemic segments of chronic preparations. Typical slow waves were not recorded in acute preparations. Absence of reflexes was noted in the post-ischaemic segment. Pendular movements were absent in isolated jejunal strips from the post-ischaemic segments. Jejunal strips from both control and post-ischaemic segments responded to transmural stimulation. The contractions were partially blocked by hexamethonium and prevented by atropine. Tetrodotoxin completely abolished these responses. It is concluded that prolonged ischaemia decreased nerve activity, abolished reflexes and produced changes in the electrical and mechanical activity of the intestines. However, the ability of this technique to produce an aganglionic and completely denervated segment is questionable.

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To my father

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INTRODUCTION

The physiology and pharmacology of the gastrointestinal tract have been studied and discussed by various authors with many discrepancies of facts and opinions. Brief as well as extensive reviews on recent advances in this field have been published (1-7). Many of the divergencies may be attributed to factors which cause difficulty in the interpretation of experimental results.

1. Gastrointestinal smooth muscle is extremely sensitive to changes in environment and experimental conditions.
2. Different regions of the gastrointestinal tract show differences in activity and behavior under similar conditions; e.g., the electrical activity and motility patterns of the stomach are different from those of the small intestines. Responses to certain drugs may also differ in the two regions.
3. Analysis of intestinal activity is further complicated by the structural complexity of the intestines. The intestinal wall is composed of several layers of smooth muscle and interactions occur between the different types of cells. Furthermore, the intestines have both an extrinsic and an intrinsic nerve supply, making complete denervation of an intestinal segment a difficult and doubtful procedure.
4. Added to this is a variety of homeostatic and reflex mechanisms which influence and control gastrointestinal activity.

ANATOMICAL STRUCTURE

The intestines have an outer longitudinal and an inner circular muscle layer. Between them are many nerve cells, axons, and ganglion synapses which form Auerbach's or the myenteric plexus. The longitudinal muscle is covered by thin serosa on the outside. Between the circular muscle and the mucosa is a thin muscularis mucosa and a submucous layer in which lies the Meissner's or the submucous plexus. Bipolar neurons in this plexus appear to have axonal connections to the mechanoreceptors and chemoreceptors in the mucosa. They also have synaptic connections to multipolar neurons in the myenteric plexus (8-10). Effects of any test action or drugs on intestinal activity could be due to a direct action on the muscle cells or an indirect action on mechano- and chemoreceptors, ganglion cells or post-ganglionic nerve endings. From the latter action either a release, or inhibition of release of mediator substances may occur. Altered mediator release then affects the muscle cells to give the observed response.

The muscle layers are innervated by both the parasympathetic and sympathetic divisions of the autonomic nervous system. The synapses in the myenteric plexus, are considered to be parasympathetic (11,12). Sympathetic innervation of the intestine is mainly postganglionic and reaches the intestine via the mesenteric nerves which accompany the blood vessels. The cell bodies of these fibres lie mainly in the coeliac and the superior mesenteric ganglia (13-15). Some cell bodies are

also found in the thoracic and abdominal sympathetic chain. The preganglionic fibres travel with the splanchnic nerves. Some of these fibres pass through the prevertebral ganglia without synapsing to reach the organs which they innervate. In the intestines the adrenergic nerves terminate in the enteric plexus around the parasympathetic ganglion cells (16-19). Recently inhibitory fibres which are probably non-adrenergic have been reported in the guinea pig taenia coli (20,21). The exact nature of these fibres and the transmitter released from them is not known. Postganglionic fibres of both divisions form a fine-meshed plexus of non-myelinated nerves[0.1μ to 1.0μ in diameter] enveloped by a Schwann syncytium, whose terminals end amongst the smooth muscle cells. This plexus has been called the "autonomic ground plexus" by some and the "terminal reticulum" by others (15-19, 22). An "intestinal cell network" first described by Cajal is also distributed throughout the muscle in close proximity to the nerve fibres. These have been shown to be quite distinct from the ground plexus and are formed of connective tissue rather than from primitive nerve cells (18). Some authors assume that the intestinal network forms a link between the autonomic ground plexus and the muscle fibres but there is no evidence to support this assumption (18,19,22). Their actual function is still unknown. Various forms of neuromuscular relationships in smooth muscle tissue have been described (12,15,16,22-27). There is no conclusive evidence to show whether or not each smooth muscle cell receives a nerve ending. Electron microscopic observations (15-19,24,26) show parts of the unmyelinated nerve fibres in

between muscle cells covered by multiple varicosities or vesicles with granules suggesting the presence of a transmitter substance which is almost certainly acetylcholine. There is no evidence of any specialization of the post-junctional smooth muscle membrane comparable to that seen in the skeletal neuromuscular junction, but some possible synaptic connections (200 \AA) between nerve and muscle can be seen (5,18,22,24). Intestinal activity may be modulated by diffusion of transmitter from a single nerve ending to a number of neighbouring cells after release (12,15,22). Alternately axons passing close to muscle fibres may not possess discrete nerve endings at all but release transmitter substances along their length thus making innervation of individual cells unnecessary (5,6,19,22). Very little is known of the mechanism of storage or synthesis of acetylcholine by the parasympathetic nerve terminals in the intestines. It is not agreed whether all of the acetylcholine is produced in the nerve terminals or, part of it is produced in the extraneuronal tissue and released from it (1,28). Furthermore, the identity of other neurotransmitters between neurones of Meissner's and Auerbach's plexuses is unknown (1,5).

INTESTINAL SMOOTH MUSCLE ACTIVITY

Smooth muscles of the intestines show spontaneous activity with fluctuations in the basal tone. The origin of this activity and its rhythmicity have been the subject of controversy for many years. These rhythmic contractions are not affected by cutting the extrinsic nerves, by nerve transmitter blocking agents or after what is considered to be

pharmacological denervation of the intrinsic nerves (29-35). However, though they may not be essential for intestinal motor activity, both the extrinsic and intrinsic nerves do play a role in regulating and co-ordinating this activity---the intrinsic intramural plexus being more important in this respect (4,36). Intrinsic reflexes initiated by local stimuli, mechanical and/or chemical, influence intestinal muscle activity. These reflexes act via the plexuses of Meissner's and Auerbach's and release inhibitor or augmentor transmitters in the muscle coats. Besides these local reflexes the entire intestinal tract is connected to the central nervous system through the vagal, sacral and splanchnic nerves, which influence intestinal activity reflexly.

Substances present in the intestinal wall and released within the gastrointestinal tract may also alter its activity. Examples are acetylcholine, 5-HT, histamine, Substance P and nor-adrenalin (37-40). Release of some of these substances may not be directly controlled by nerves and each may act directly on the muscle or by causing release of the transmitter substance. In addition to substances intrinsic to the intestine, catecholamines from the adrenal medulla, posterior pituitary hormones, or substances such as histamine, bradykinin, etc., released from other sites may affect intestinal motility.

In the past relatively few investigations on the electrical activity of the intestines have been done. Isolated preparations of guinea pig taenia coli and intestinal strips were used, and a few in vivo studies on the cat and rabbit intestines were also done (32,41-46). Recently, with renewed

interest in this field electrical activity of the intestines of various species, its relation to contraction and effect of drugs on it have been studied extensively (4,33,36,47-56).

Effect of drugs on intestinal activity of the dog is of interest because of the similarity in behavior of this organ in the dog and in the human being (33,36,49,50). Due to its size and thickness in vitro studies of drug action on the electrical or mechanical activity of the whole intestine is difficult and of limited value. Ahlquist and Levy used intravenous injections of adrenergic drugs to study adrenergic receptors of the intestines in anaesthetized dogs (58). Intravenous injections of adrenergic drugs cause alterations in cardiovascular parameters and evoke homeostatic reflexes which may obscure the direct effect of drugs on intestinal activity. It has been my object in the first part of this thesis:

1. To study and confirm the reports of other workers regarding the nature of the electrical activity of the intestines in the dog and its relation to mechanical activity.
2. To study the adrenergic receptors in the intestines and their possible location, using intra-arterial perfusions to avoid the homeostatic reflexes.
3. To find out if cocaine potentiated the effects of catecholamines in the intestines as is reported in some tissues (59, 60).

The purpose in the second part of my thesis is:

1. To study the effect of ischaemia on the electrical and mechanical activity of the small intestine of the dog.
2. To relate these effects to nerve or muscle damage.
3. To find out if ischaemia of 4 hours duration by the technique of Hukuhara, effectively destroys the ganglion cells and their axons in the myenteric plexus.

PART 1. ELECTROPHYSIOLOGY OF THE SMALL INTESTINES

A. Recording Techniques

Early investigations of intestinal activity were carried out by visual observations of exposed intestines or by means of enterographs and enteromyographs. Bayliss and Starling were among the first to record the separate activities of the longitudinal and circular muscle coats (61,62). They recorded rhythmic pendulum movements and peristaltic contractions and showed that the occurrence of neither of them required intact connections with the central nervous system.

(1) Extracellular Techniques: Records of electrical activity before 1935 were obtained with string galvanometers and without adequate amplifications. Potential changes of slow frequency were recorded more or less correctly but the galvanometers did not have a high enough frequency response to record potentials of relatively high frequency. RC or AC amplifiers connected to various recording instruments permitted recording of high frequency changes but the slow potentials were not accurately recorded. Furthermore, simultaneous recording of electrical activity and mechanogram was not done so that any time relation claimed between the two was more or less arbitrary.

Alvarez and Mahoney (29,30) recorded electrical potential changes accompanying rhythmic contractions. These potentials had a characteristic wave form and a definite relation was seen between this wave and the mechanical movements

of contraction and relaxation. They assumed that these potential changes were muscle action currents and that the same principles were involved in the electrophysiology of smooth muscle and skeletal muscles. Puestow (31) demonstrated reflex secretory and motor activity in excised and exteriorized intestinal segments in the dog and showed these responses were at least partially dependent on nerves. Both he and Berkson (42-44) recorded monophasic and biphasic potentials consisting of a slow component and rapid spikes appearing during contractions. This electrical complex was not affected by denervation or by nicotine. Berkson pointed out that the concept of muscle action currents implies a necessary association between action and currents which was not always true in the intestinal smooth muscle activity. Complete dissociation of the electrical and mechanical activity sometimes occurred in apparently normal loops. These authors concluded that the electrical potentials originate probably within the intrinsic nerve net.

Electrophysiology of various visceral smooth muscles was studied extensively by Bozler, (32,45,63-66). Simultaneous recording of the electrical and mechanical activity was done using a direct coupled amplifier and oscillograph, and an isometric lever to record the mechanical activity. He produced an inactive region in the intestines of rat and guinea pig by pushing part of the segment into a narrow glass tube damaging it from pressure and making it anoxic (64). Recording the potential difference between these two regions produced

interpretable monophasic records consisting of a slow potential variation and fast spikes. He showed that the configuration of the monophasic slow wave can be derived mathematically from differential records obtained by recording the potential difference between closely approximated surface electrodes. Ambache (46) recorded similar types of potentials and called the slow component "A" wave and the fast spikes "B" wave. He postulated that the fast spikes of the "B" wave were action potentials originating from a synchronous group of muscle fibres and the "A" wave originated from discharge of impulses which may arise from the nerve net.

Recording Apparatus: The direct coupled systems with either a cathode ray oscilloscope or with a d.c. pen writer systems are now the conventional recording apparatus used, and appear adequate to record all of the known electrical activity of the smooth muscles of the gastrointestinal tract. Limitations in the use of this system are briefly reviewed by Daniel and Chapman (4).

Electrodes: A variety of types of electrodes have been used in the past years. Alvarez and Mahoney used Zn Zn sulphate and calomel electrodes and Bozler used Zn Zn sulphate, silver wire and cotton wicks for his monophasic recording. For his differential recording he designed a special electrode (67). It consisted of 2 capillary calomel electrodes (0.3 mm inside diameter) enclosed in glass tubing and arranged so that the distance between the two electrodes was 0.6 mm. Metal electrodes polarize in solution to a variable extent. Chlorided silver and platinum electrodes are more suitable for use as recording

electrodes, and are called "nonpolarizable". Their polarization potentials are relatively constant under conditions for which they are normally used and small compared to the phenomenon under study.

In monopolar recording the active or recording electrode is placed in the area under study and potential difference is measured against an indifferent electrode placed in the bathing medium, or in some distant part of the animal chosen to give minimum recording of cardiac electrical activity. This technique usually gives monophasic records. Biphasic records are obtained when some other component such as a field potential of the bathing solution or volume conductor is involved (52). In bipolar recording technique, both electrodes are placed in the area under study and the potential difference between the two is measured. This gives a biphasic wave the configuration of which depends on the distance between the electrodes and their orientation. For in vitro studies, the tissue is usually suspended in a suitable bathing medium. Electrical contacts may be made with cotton wicks, glass pipettes or simply through the bathing medium. Daniel et al. (4,33,50) in their extracellular, in vivo studies, threaded thin silver wire under the serosa or sewed them into the intestinal wall and recorded the intestinal activity for prolonged periods. Armstrong et al. (47-49) used copper wire or plain hypodermic needles and Bass et al. (55) used solid needle electrodes inserted through the skin of an exteriorized Biebl loop to study the electrical activity in the duodenum. They also used punctate monopolar electrodes for studies of the electrical activity of the stomach and the gastroduodenal junction (56).

(2) Intracellular Recording: The use of microelectrodes for intracellular studies of smooth muscle potentials was introduced by Bulbring and Hooton (68,69) and has since been applied to a variety of smooth muscles. Longitudinal muscles of guinea pig intestines and taenia coli were studied using microelectrodes designed by Ling and Gerard (70,71) for the study of striated muscles. These were glass capillaries drawn out to tips of $< 1.0 \mu$ diameter and were not suitable for the study of smooth muscle cells. These cells ($4-7 \mu$) were smaller than striated muscle cells and more likely to be damaged during impalement. Even using electrodes with tips less than 0.5μ ; (resistance around $35-40 M\Omega$) muscle movements due to spontaneous activity made it difficult to remain within the cell or to record true potentials. The values obtained were low compared to other excitable tissues, fluctuated over a wide range (25-75 mv) and slowly decayed (72-74). In the sphincter pupillae the membrane potential was reasonably maintained around 60 mv. In the intestines, the potential varied with stretch (43-60 mv) and decayed at varying rates (73,74). Holman (75) attempted to minimize the degree of cell injury by using high resistance capillary microelectrodes ($35-70 M\Omega$) with tip potentials < 5 mv. Values similar to those of Bulbring (72) were obtained for the guinea pig taenia coli. She recorded slow waves of 1-10 mv and spikes of 50-75 mv. and studied the effects of changes in the external ionic concentrations on electrical and mechanical activity.

The popular technique of intracellular recording now used is a modification of the "floating" microelectrode technique

used for the heart (52,76). A microcapillary electrode filled with 3 M KCl is suspended from a micromanipulator by approximately 3 cm of 1 mil tungsten or platinum wire. The suspended or "floating" microelectrode, unlike the rigid electrode is able to move with the movements of the muscle and the probability of the electrode being pulled out of the cell or damaging it was less.

In spite of attempts at perfection, technical difficulties are still involved in obtaining reliable records.

1. Electrodes with diameters less than $0.25\ \mu$ are fragile and can be easily broken.
2. They are also easily blocked by tissue constituents.
3. Attempts at studying R.M.P. of smooth muscles is complicated by the presence of tip potentials (77).
4. It is extremely difficult to minimize muscle movements relative to the electrode and the cells themselves are sensitive to mechanical deformation.
5. Penetration of smooth muscle cell by microelectrodes has not been shown microscopically, and one has to rely on electrical recording to assess whether impalement is satisfactory.
6. There is no direct method of assessing the degree of injury to the cell during impalement or whether the electrode has been "sealed" into the cell satisfactorily. The magnitude and stability of the measured potential is the only indication of the degree of cell trauma.

Though the use of intracellular recording technique is difficult and interpretation must be made cautiously, it has become possible to obtain reproducible results of resting potential and action potentials in smooth muscle.

(3) Sucrose Gap Technique: Stampfli described the Sucrose Gap technique for measuring the resting membrane potential of nerve fibres with external electrodes (78). He calculated that in

biological core conductors the true value of the membrane potential measured with external electrodes was in fact reduced by a short circuiting factor $\frac{r_1}{r_1 + r_2}$ where $r_1 + r_2$ are the longitudinal resistances of the external and internal medium per unit length respectively. He therefore increased the outside resistance r_1 of his preparation in the interpolar region by replacing most of the ions in the intestinal fluid with nearly ion-free sucrose solution. The apparatus consists of a T.-tube in which isotonic sucrose solution of at least $2 \times 10^6 \Omega \text{ cm}$ specific resistance enters through the base of the tube at a constant rate. The horizontal arms contained the nerve fibres and the sucrose solution exits at the two sides. The ends of the nerve fibre on either side are passed through a pair of vertical tubes through which flows Ringer solution on one side and Ringer's solution or some test solution (usually a high potassium depolarising solution) on the other. Ag. AgCl electrodes make contact at the lower ends of the vertical channels and the potential difference between the two ends of the preparation is measured. Short circuiting between the electrodes is minimized by the central stream of high resistance sucrose solution. Consistent values of resting membrane potentials of myelinated and nonmyelinated nerve fibres were obtained by this method (79,80), and the records obtained were similar to those obtained by the intracellular technique. Burnstock and Straub (81) and Bulbring et al. (82) applied this method to smooth muscles, assuming that smooth muscle cells behave like a core conductor when mounted between two recording electrodes. Smooth muscle strips from guinea pig taenia coli and longitudinal muscles of the intestines were studied. Resting membrane

potential value was similar to those recorded by others (72-74) with intracellular microelectrodes. Smaller spikes were recorded with the sucrose gap technique but changes produced by drugs and ions could be effectively studied. Both regular and irregular spikes of around 14 mv were observed. This was less than the height (30-60 mv) of spikes from single fibres recorded with intracellular microelectrodes (72-74). Burnstock et al. attributes this to temporal dispersion of individual spikes and to the occurrence of inactive fibres in the area. In this technique, a larger number of fibres contribute to the values obtained. This method is now widely used for in vitro studies of membrane potential changes in many smooth muscles (83,84).

(4) Pressure Electrodes: These were used by Bortoff (52,53) to study the electrical activity and conduction in isolated cat intestines. The capillary glass electrodes are of a larger diameter than those used for intracellular studies (3 m.m. od). These are drawn out to tips averaging about 0.5 m.m. and filled with Agar-tyrode solution. These electrodes were connected to the input of a d.c. amplifier by chlorided silver wire and recorded by a polygraph. Bipolar electrodes made contact with the tissue by cotton wicks. With the electrode exerting some pressure (1-2G) on the tissue, the configuration and polarity of the electrical potentials recorded resembled those obtained by intracellular microelectrodes though they were of a smaller amplitude. These electrodes were attached to a force transducer and this arrangement permitted simultaneous recording of electrical and mechanical activity from the same area of the intestines. (see later).

(B) The Electrical Activity

A number of investigators have studied the electrical activity of the small intestines of the dog (4,31-33,36,42-44,46, 47-51,55,57), the cat (33,52-54), rodents (44,63, 66) and man (4,33,50). It is now generally accepted that spontaneous electrical activity of the mammalian small intestines consists of two components, a slow periodic potential variation and fast spike potentials. The fast spikes are usually referred to as "action potentials" or "spike potentials" and the slow component as "basic electrical rhythm"(55,56) as pacemaker potentials (54) in intracellular records or simply as the slow waves (31,32, 50-54). Slow waves can be recorded in the absence of visible contractions (31,44,46,48,51,55) and were the same during inactivity and during contractions. The form of the complex was not altered by section of the extrinsic nerves or by moderate doses of drugs which stimulate or inhibit intestinal motility, e.g., acetylcholine, 5-HT or adrenalin (4,33,44). They are not abolished by blocking agents like atropine or phenoxybenzamine or pronethalol (see later) in their effective concentrations (33,50) or by nicotine, hexamethonium and cocaine in doses which normally interferes with nerve conduction (4,36,50). They are however sensitive to changes in temperature, a decrease in temperature decreasing the amplitude and frequency of the slow waves (50). Slow wave frequency and amplitudes are also affected by larger doses of adrenergic and cholinergic drugs (33,53). Anoxia or ischaemia for short periods abolishes the slow waves during the period of asphyxiation or ischaemia. They recover with varying rapidity when the oxygen or blood supply is re-established (85).

The size, and frequency, of the slow waves vary slightly in different regions of intestinal tract in the dog (4,33). In the duodenum and the jejunum, the slow waves are regular, with greater amplitude and a frequency of 15-21 per minute. In the ileum, the slow waves are less regular both in size and frequency which is about 7-11 per minute (4,33) in the terminal part. They are smaller with a slower rate of positive deflection consequent to a slower rate of conduction in the ileum. They appear to spread distally with velocities of 10-20 cm/sec. in the duodenum, 6-10 cm/sec. in the jejunum, and 0.3-1 cm/sec. in the terminal ileum (47-49,4,33) where the slow waves appear indistinct and spread out in time.

The configuration of the slow waves also show slight variations depending on recording techniques and the recording site. With monopolar recording techniques, extracellular records from the upper small intestine of the dog show a sharp positive deflection, followed by a slower negative deflection, appearing as a plateau, and finally a faster return to the original potential. When there is activity either spontaneous or induced, a varying number of predominantly negative spikes appear superimposed on the slow waves. These fast spikes are associated with contractile activity and the frequency of their appearance is proportional to the intensity of the contraction. When recorded with sufficiently small electrodes they can be shown to correspond to action potentials of individual smooth muscle fibres having an amplitude of 0.1-2 mv. (4,33). In normal spontaneous activity, spike potentials and contractions do not accompany every cycle of the slow wave. Also drugs which abolish the spikes usually

have little or no effect on the frequency of the slow waves e.g., atropine, adrenalin (54,55). Many believe that the slow waves changes the excitability of the muscle and regulate the occurrence of spikes and attendant contractions in both the longitudinal and circular muscle layers (48,55).

When spike potentials appear either singly or in bursts, they tend to occur in phase with the slow waves (when the muscle electrode is positive in relation to the indifferent electrode). Spikes out of phase with the slow waves are noted when the muscle activity is irregular as during spasms or when pressure is applied. In normal unanaesthetised (36,49,55) and anaesthetised (4,49,50) dogs, spikes out of phase with slow waves are rare and when present are followed by forceful contractions. In extracellular records, spikes are usually seen at the plateau of the slow wave but may sometimes be seen at the peak or in the troughs. In intracellular records, they occur at peak depolarisation.

Study of smooth muscle transmembrane potentials with intracellular microelectrodes has contributed much to the interpretation of phenomena recorded extracellularly. Such studies show that spontaneous activity of the intestines is related to intrinsic instability of the smooth muscle membrane potential and the slow waves represent periodic almost sinusoidal depolarisations of the longitudinal muscle cell membrane (51-54). When these reach threshold depolarisation (-24 mv to -32 mv) they generate an all or none spike which appears at the peak of the slow wave. Spikes may appear

singly, or as multiple spikes in a single slow wave, and may be full size with overshoot or abortive. When the depolarisation is not sufficient to generate spikes miniature potentials may be seen on the slow waves (52). Both the instability of the membrane and the periodic depolarisations are a fundamental property of the longitudinal muscle cells of the intestines (51). The resting membrane potential recorded is around 40-60 mv (51,52,75) and shows slow fluctuations. The amplitude of the slow waves is 10-15 mv and that of the spikes is 50-75 mv (51,75,86). These values are low when compared to other excitable tissues (skeletal muscle - 90 mv) and may be due to damage of the small muscle cells during impalement or to a difference in ionic permeability of the smooth muscle cell membrane. Each spike is preceded by a pre-potential of 1-2 mv which is described by some as a pacemaker potential.

Bortoff showed that slow waves recorded from cat intestines with glass capillary pressure electrodes (0.5 mm) assumed different types of configuration depending on the recording technique -- monopolar or bipolar, the position of the recording electrode, and the pressure exerted on the tissue (52,53,87). When the recording electrode was in contact with the tissue and exerting pressure (1-2G) on it a pure monophasic slow wave was obtained, which resembled the intracellularly recorded slow wave both in configuration and polarity, but was smaller. The slow wave recorded under such conditions, he said, represented the time course of the changes in the membrane potential associated with the intracellularly recorded slow wave. When

the recording electrode was in the bathing medium, but 1-2 mm away from the tissue, the recorded slow wave was the "field potential" and consisted of an initial positive deflection, followed by a rapid negative swing and a graded return to the original potential. This configuration resembled the second derivative of the transmembrane potential change and represents the time course, direction and density of the membrane current associated with the potential changes. A third configuration was seen when the electrode was touching the tissue but exerting no pressure on it. This type consisted of a rapid positive deflection with a negative notch, followed by a prolonged positive phase and a gradual return to the base line. Bortoff describes this as a superposition of the monophasic wave and its field potential and it therefore represents the transmembrane potential as well as the membrane current associated with the potential.

Configuration of the slow wave obtained with bipolar wick electrodes depended on the distance between the electrodes and their orientation. When the distance between the electrodes was less than 2 mm. the slow wave recorded was similar to that described by Bozler (32) and approximates the first time derivative of the monophasic wave. When the distance between the two electrodes was > 6 cm. the resultant slow wave is the sum of two slow waves recorded with opposite polarity (4). These configurations are of the type obtained from core conductors under the same condition and the assumption was that the flow of current associated with depolarisation in smooth muscle of the intestine must be similar to that in a core conductor (4,52).

(C) Origin and Propagation of Slow Waves and Action Potentials

The majority of investigators of the electrical activity of the intestines agree that fast spikes are the action potentials from the muscle cells but the origin, function and propagation of slow waves has been the subject of controversy. Alvarez and Mahoney thought they were muscle action currents (29). Berkson and Puestow suggested they were possibly neurogenic (43,31). Ambache stated that they may arise from a nerve net or from the interstitial cells. Bozler carried out extensive studies on visceral smooth muscles of various species of animals and published reviews on the subject (63,66). He gave evidence against the neurogenic theory of origin of the slow waves and his reasons for considering that they were myogenic. Recent investigators agree that both the slow waves and the action potentials are myogenic though Hukuhara in his recent paper suggested that rhythmic spontaneous activity of the intestines might be neurogenic (88). The configuration and magnitude of the slow waves are not affected by nicotine or curare or by changes in the external Na concentration (52,75) which they would be if they were neurogenic.

Daniel (33) and Holaday (36) using glass electrodes, recorded slow waves and action potentials from different depths in the intestinal wall. The amplitude of the slow waves was almost independent of the depths from which it was recorded, but the amplitude of the action potentials decreased as the electrode was pushed deeper into the tissue. They suggested that slow waves and action potentials might originate independently from

different areas in the intestinal wall. Studies with intracellular microelectrodes later (51) showed that the slow waves represented periodic depolarisations of the longitudinal muscle cells and this was inherent to the intestinal muscle. These were often accompanied by action potentials. The independence of appearance of slow waves and spikes in many electrical patterns and their different behavior in response to drugs and ionic effects favours the idea that they originate differently. Recently Tamai and Prosser (54) have shown that spikes are more sensitive to reduced resting potential, high external K concentration, low external Na concentration and low external Ca concentration than the slow waves. It was postulated that they were produced in different molecular types of membrane within the same cell and that the ionic mechanism that underly the different electrical activities are quantitatively different.

Studies with multiple electrodes placed at varying distances in the long axis of the intestines indicate that slow waves in the longitudinal muscle are propagated. Spikes are either not propagated or are propagated only a few millimeters. Recording electrodes from sites a few centimeters apart may record spike potentials from one site and not from another. When two adjoining areas are generating spikes, the number, duration and amplitude are often different (4,57). Slow waves are propagated for longer distances with a velocity of 10-20 cm/sec. in the duodenum and jejunum and 0.3-1 cm/sec. in the ileum. Alvarez et al. assume this difference in propagation velocity to be due to the existence of a metabolic gradient in the intestines. Others suggest that there is a pacemaker

area in the duodenum possibly at the entrance of the bile duct which "drives" the slow waves in distal portions of the duodenum, jejunum and ileum (49). While slow waves which originate in the upper duodenum may influence the rate of the slow waves in the lower duodenum and jejunum, every cell in the intestinal tract is capable of generating slow waves when continuity is interrupted by section or when distal propagation has been interfered with by pressure, clamping or infiltration with cocaine. When propagation is blocked, the slow waves in the duodenum or jejunum just distal to the section or affected area can still be recorded but at a slower frequency. In the ileum the slow waves are apparently independent of duodenal influence and the rate and amplitude of the slow waves in the lower ileum are practically unaffected by clamping or separation from the pacemaker area (4).

Propagation of slow waves under normal circumstances takes place in the caudad direction. Dissociation and oral spread has been occasionally reported (36). This generally occurs when the usual pattern of spread has been disrupted and slow waves originating in the lower healthy region spread in the opposite direction. Bass et al. (55) with electrodes in the radial axis showed that slow waves were more or less synchronized at all points in the cross-section of the intestines. Kobayashi et al. (89) in their recent paper showed a difference in time (180-260 msc) in the rate of rise of the slow waves recorded from 2 electrodes 9-10 mm apart. He suggested a "multiple pacemaker loci" together with conduction in the underlying circular layer.

Circular muscles are not spontaneously active when isolated from other intestinal tissues and comparable slow waves cannot be recorded from ganglion free circular muscle fibres of the cat small intestine (90,91). Isolated circular muscle in form of strips or rings respond to shock or chemical stimuli with small spikes which are transmitted in the long axis of the fibres at about 4 cm/sec. Slow waves of smaller magnitude can be recorded from the circular muscle if it is attached to the longitudinal layer (52,87). Extracellular and intracellular records from the circular muscle, from which a piece of longitudinal muscle layer has been cut show typical slow waves when the electrodes were placed at a short distance from the cut edge of the longitudinal muscle. [<12 mm from the lateral edges and <3 mm from the oral or aboral edge (87)] . Their amplitude decreases as you move further away from the cut edge. It was concluded that the slow waves did indeed originate from the longitudinal muscle layer and transmission to the circular layer was affected by electrotonic spread (87,89). Spikes initiated by drugs or electrical stimulation pass in both direction between the layers with similar latencies. Spread of both the slow waves and spikes between the two layers is not affected by blocking agents like nicotine, hexamethonium procaine etc. and probably does not involve the Auerbach's plexus. It has been claimed that strands of muscle and connective tissue can be seen histologically in between the two layers and that transmission can be stopped by cutting between the layers (89).

Lateral and spiral spread of spontaneous and induced spikes have also been observed in isolated circular muscle. If a segment is partly slit so that two rings are formed, connected by a bridge of muscle, transmission takes place along this bridge in one or both directions depending on local excitability. Transmission occurs even when two rings are completely cut and pushed close together (90).

The mechanism by which spread of electrical activity occurs in either layer of muscle is not obvious. It does not involve the extrinsic nerves and the intrinsic plexuses though the participation of a nerve net has not been thoroughly excluded. Evidence against "mechanical pull hypothesis" is provided by the fact that strips of circular muscles do not respond to brief stretches (92) and by conduction over cut or immobilised areas (90,91). Conduction by chemical synaptic transmission between muscle fibres is also declared unlikely. Agents known to block transmission across various synaptic junctions have little or no effect on propagation velocity in the intestines. (24,90,91).

Electrical transmission appears to be the most likely mechanism for propagation of electrical activity in intestinal muscle cells. Reviews on this subject have been published (24,95,96). Bozler postulated transmission by means of protoplasmic syncytium or functional continuity between muscle fibres (66). Electron microscopic studies (24,93-95) of intestinal muscles have failed to show any evidence of protoplasmic continuity between fibres. However "intercellular bridges" have been reported in the form of areas of close

contact between adjacent cells with protuberances from both cells meeting, or from one cell inserting into a pocket of another cell (93). There is much dispute in literature regarding these areas of contact between the bridges. Some workers claim these processes are spanned by intact membranes (93). Others consider the membranes incomplete with protoplasmic continuity (97). Prosser, Burnstock and Kahn (93) argue that the resistance across such a narrow bridge with or without membranes would be high (above 10^8 ohms) and it seems unlikely that these bridges would represent "low resistance pathways" between adjacent fibres. However the specific resistance of the membrane across the bridge could be relatively lower than the total membrane resistance of other parts of the cell. If that were so, then these bridges could be the electrical pathway between adjacent cells. Dewey and Barr (97,98) called these bridges "Nexuses" and showed that these areas of close contacts were not just a juxtaposition of membranes of two adjacent cells but there was actual fusion with no intervening extracellular space. These were of different sizes and have been reported in several tissues including the human oesophagus and the intestine of the rat and the cat. He suggests that propagation of electrical activity occurs by electrotonic spread over these regions of relatively low resistance.

Another type of electrical transmission that has been proposed in intestinal muscle cells is "ephaptic". This is transmission occurring across an intercellular gap, between discrete smooth muscle cells. The spread of spikes across two separate rings of circular muscle in close contact, and the observation with electron microscopy of discrete cells in

some organs tends to favour this theory (24,98). No conclusive evidence has been published, whether or not ephaptic transmission does occur, or whether spread is electrotonic over some form of low resistance pathways. Dewey and Barr (96,97,98) are in favour of the nexus theory and Prosser (91) suggests that it also requires summation of overlapping electrical fields from many parallel fibres.

The Ionic Basis of Electrical Activity

Very little is known of the ion movements underlying electrical activity of smooth muscle cells. The role of electrolytes in the electrophysiology of smooth muscles of different organs and different species has been discussed by a number of authors (3,86,99-102). An extensive review covering the uterus, gastrointestinal tract and the vascular smooth muscle has also been recently presented (103).

Studies of various smooth muscle organs indicate that:

1. Distribution of physiologically active ions and their transmembrane gradients in smooth muscles differs from those of other excitable tissues.
2. The electrical properties of smooth muscle cells do not conform to the ionic theory of origin of the resting and action potential applied to nerve and skeletal muscles.

It has not yet been possible to measure the intracellular ion concentrations directly. Measurements of the total tissue water and electrolyte concentration, as well as the water and electrolyte concentration in the extracellular space are carried out. The value of intracellular electrolyte concentration is then calculated (3,86). The different methods of

measuring the extracellular space, the difficulties involved and the assumptions that have to be made in carrying out this procedure have been discussed (3,86,101,103). Different values of extracellular space assumed by investigators using different methods of estimation have resulted in variation in published values of intracellular ion concentrations. However it is a consistent finding that concentration of Na and Cl in various types of smooth muscles is higher than in skeletal muscles (3,100,103,104). The Na concentration in dog small intestine is about 54.5-65.3 meq/Kg wet weight and around 35 meq/Kg wet weight in skeletal muscles (102). The K concentration on the other hand is relatively low, ranging from 53.6-78.2 meq/Kg wet weight in the intestine and usually around 100 meq/Kg wet weight in the skeletal muscle. The Cl concentration in the intestine is about 53.3 - 54.5 meq/Kg wet weight and in the skeletal muscle is usually 10-20 meq/Kg wet weight.

The Resting Potential. The distribution of ions on either side of the cell membrane and the relative permeabilities can account for the membrane potential in most excitable tissues. In nerve and skeletal muscles the resting potential is ^{approximately} determined by the diffusion potential for K. The membrane potential can be expressed quantitatively by the Goldman equation (105).

$$E = \frac{RT}{F} \ln \frac{P_K [K]_i + P_{Na} [Na]_i + P_{Cl} [Cl]_o}{P_K [K]_o + P_{Na} [Na]_o + P_{Cl} [Cl]_i}$$

where $[K]_i$, $[Na]_i$ and $[Cl]_i$ are the activities (concentration x activity coefficients) of the ion inside the cell and $[K]_o$, $[Na]_o$ and $[Cl]_o$ are the activities of ions outside the cell and P_K , P_{Na} and P_{Cl} are the permeability constants. At high $[K]_o$, because the

K conductance is high and Cl is assumed to be passively distributed, the membrane potential corresponds to the equilibrium potential for K (E_k) given by the Nernst equation (106)

$$E_k = \frac{RT}{F} \ln \frac{[K]_i}{[K]_o}$$

The membrane potential of smooth muscle is also partly a K potential. In skeletal muscles the slope of the line expressing the relation between membrane potential and $\log [K]_o$ corresponds to that predicted by the ionic theory. In smooth muscles, this same slope is lower over the whole range of $[K]_o$. Boyle and Conway (107) and Hodgkin and Horowicz (108) have shown that in skeletal muscles, in $[K]_o$ exceeding 10 mM, K and Cl exist in equilibrium. If $[K]_o \times [Cl]_o$ was held constant, changes in $[K]_o$ produced changes in membrane potential predictable by the Nernst equation for K. In smooth muscles on the other hand, the line relating membrane potential to $\log [K]_o$ had a slope of 33 mv per tenfold change in concentration, instead of 58-60 mv predicted by the ionic theory (81,109,110). This suggested the participation of some other factor in determining the membrane potential. It has been pointed out (111) that, in the above studies, $[K]_o \times [Cl]_o$ was not kept constant. If $[K]_o \times [Cl]_o$ was held constant, as done by some workers (111-113) the slope obtained for a tenfold change in $[K]_o$ was close to the value predicted by the Nernst equation.

Smooth muscle cell membrane is more permeable to Na than skeletal muscle, and Na fluxes have been shown to be very rapid. The Na conductance is also very high though it is not certain what proportion of the Na flux contributes to the membrane conductance. K flux on the other hand is relatively low. The high Na conductance and possibly the high Cl conductance (since substitution of nondiffusible anion $SO_4 =$ for Cl

produces depolarisation) has been postulated to be responsible for the low and unstable membrane potential (103).

The Action Potential. According to the hypothesis of Hodgkin and Huxley (106) the action potential in nerve and skeletal muscle is brought about by a transient and specific increase in Na conductance. This results in ion movements down their electrochemical gradients using the Na carrier system which depolarises the membrane. This is followed by inactivation of the Na carrier system and together with a delayed increase in K conductance, produces repolarisation. A great deal of evidence has recently accumulated which opposes this hypothesis in smooth muscle activity. There is no clear relation between rate of rise and amplitude of the action potential and the external Na concentration, $[Na]_0$ (99,101,103,110,114,115). Spikes in smooth muscle persist for long periods even when $[Na]_0$ is reduced to 1/9 of normal $[Na]_0$ (101). Attempts have been made to explain this finding with the assumption that there may be only a limited number of Na carriers or permeation sites available in smooth muscle membrane (99,101, 103) or that the Na carrier mechanism is not specific. However the fact that tetrodotoxin abolishes the spikes in nerve and skeletal muscle but not those in smooth muscles (116,117) suggests a different mechanism not involving increased Na conductance for spike production in smooth muscle. What in fact produces the depolarisation and repolarisation, and the ions involved is as yet unknown. Ca seems to play an important part in the activity of smooth muscles. The maximum rate of rise of the action potential varies with the external Ca concentration $[Ca]_0$ (99,103). Spontaneous spikes continues in Na-free media only if Ca is

present and removal of Ca results in cessation of spontaneous activity, which returns when Ca is added (103). Ca is known to stabilise the membrane and control Na permeability. It has been suggested that Ca alters the smooth muscle cell membrane and increases the number of Na sites available for activation (99,103). The dependence of the smooth muscle action potential on the presence of Ca, more than on other ions has led to the postulation that the Ca ions might carry the inward current or at least substitute for Na (114). This has been shown in the crustacean muscle. The results with tetrodotoxin appear to support this hypothesis. Hagiwara and Naka (118) had stated that tetrodotoxin had no effect on the production of the action potential in barnacle muscle fibres and the ions entering during the spike, were Ca. However conclusive evidence in this field is still lacking.

The Slow Waves. Ion movements underlying production and propagation of slow waves has been studied by Daniel using intra-arterial perfusions of Krebs Ringer solution with altered electrolyte concentrations, or with added metabolic inhibitors (102). The periodic depolarisation of longitudinal smooth muscles which causes the slow waves were shown to be extremely stable to alterations in the external ionic environment. Reduction of Na^+ , K^+ or Cl^- concentrations did not seriously alter slow waves, though the electrolyte concentration in the muscle was markedly altered. The slow waves were shown to be depressed or abolished by metabolic

inhibitors and substances which inhibit active transport such as lithium ion or ouabain. This effect was diminished but not abolished by reserpinisation which indicates that part of this action was due to release of catecholamines which in turn depressed the slow waves. However part of the action is unaffected by reserpine and was probably a direct action resulting from inhibition of active ion transport. Daniel suggested that the depolarisation of the slow waves are not the result of altered permeability of the cell membrane to the principle ion present in the cells and the intestinal fluid. He postulated that the existence of the slow waves depended upon the function of the electrogenic pump. It is possible that the slow waves are caused by the oscillatory activity of such a pump. This theory leaves unexplained the mechanism of propagation of the slow waves.

II. MECHANICAL ACTIVITY OF THE SMALL INTESTINES

(A) Recording Techniques

Intestinal motility has been studied by clinicians and radiologists using X-ray screens and serial photographs. Physiologists and pharmacologists have investigated this activity by studying the progress of a bolus down the lumen or in vitro by suspending isolated tissue in an organ bath and recording by means of a writing lever and a smoked drum. Intestinal movements have also been studied either in vivo or in vitro by introducing small condom balloons into the lumen inflated with air or filled with water and recording changes in intraluminal pressures. Balloons are attached to appropriate transducers and fed into the input of a d.c. amplifier system. These methods yield only a crude mean of the contractile activity of the whole area in contact with the balloon. Technical problems and limitations in the use of this technique have been pointed out by Code et al. and others (119-122). A large balloon, records the mean of changes in intraluminal pressure of the segment. Contractions from one area may balance small relaxations present in the adjacent area. It has also been pointed out that the presence of the balloon may influence the activity recorded by distension of the intestine. High pressure in the balloon cause stretching of the muscle. In addition, the balloon itself is elastic resisting inflation and can also exert pressure. Alternately if the balloon pressure is too low it may collapse wholly or partially, so that a portion of the response is not recorded.

Jacoby et al. (122) have modelled an extraluminal

contractile force transducer for recording gastrointestinal motility in vivo. The principle is based on measurement of strain in a small metal strip completely enclosed within a physiologically inert silicone rubber and which could be attached to two points in the intestine by surgical sutures. When placed along the long axis of the intestine it apparently records mainly the contractile activity of the longitudinal muscle and when placed in the transverse axis, it apparently records mainly the activity of the circular muscles. Using these transducers in both axis at the same level of the intestine, separate and simultaneous recording of the activity of the two muscles in the same area appears to be feasible.

(B) Types of Mechanical Activity.

Two main types of movements have been described in the intestine. The mixing or segmenting movements, called the pendulum movements, are rhythmic spontaneous contractions propagated for very short distances. These are not affected by section of the extrinsic nerves. Their function is mainly to mix the intestinal contents and they do not cause a progressive forward movement of a bolus or other intestinal contents (62). Anoxia or ischaemia of short duration as well as drugs, such as adrenalin, abolishes these movements for short periods (1,5), but they recover with varying rapidity when sufficient oxygen or blood has been supplied or when the drugs have been removed.

The second type of movement described, is propulsive and caused movement of a bolus down the intestinal lumen. They as well as pendular waves contribute to the recorded spontaneous

activity which may be accompanied by spikes in the simultaneous electrical record. These peristaltic waves are variable. Some may progress only for a short distance. When there is excessive stimulation by local factors or reflexly produced, a contraction wave passes virtually over the entire length of the small intestine at a much faster rate; "mass peristalsis". In some species e.g., the rabbit, this is said to be a common occurrence (65).

Unlike the pendular movements, peristalsis depends on the existence of intrinsic nerves and co-ordinated local reflexes (4,5,123-127). The effective stimulus for this reflex is usually distension and/or stretch but can be elicited by stimulation of mucosal receptors mechanically or chemically. These receptors are postulated to convey impulses to the cells in the Meissner's and the Auerbach's plexuses. The intestines respond with contraction first of the longitudinal muscle resulting in shortening of the segment (preparatory phase). This is followed by a contraction of the circular muscle and a wave travels aborally while the longitudinal muscle relaxes (emptying phase) (5). It has been shown that although the two phases follow a fixed pattern, the activity of the two muscle layers is independent (125). The early response of the longitudinal muscle is insensitive to hexamethonium and local anaesthetics and it has been claimed by Hukuhara et al. that it is not a true peristaltic reflex but is a consequence of the visco-elastic property of the intestinal wall (123,124). High concentrations of acetylcholine or

histamine caused a non-specific block of the longitudinal muscle contractions, enabling the activity of the circular muscle in the emptying phase to be studied separately (125). The absence of the preparatory phase did not interfere with the efficacy of the emptying phase. The contraction of the circular muscle in that phase was prevented by hexamethonium, tubocurarine, and local anaesthetics. This indicated the participation of the ganglion cells of the myenteric plexus, the reflex arc involving at least one cholinergic synapse. When the preparatory phase was elicited alone or when the intestinal filling was below threshold, the longitudinal muscle response was graded (119,126). Kosterlitz et al. calls this type of response type I contractions. Fast rhythmic contractions of the longitudinal muscle also occur during the peristaltic reflex proper. This has been called the type II contractions and appears superimposed on the "slow graded" reflex. These fast contractions are blocked by hexamethonium. Since the graded response is not affected by hexamethonium and other ganglion blocking drugs, it has been suggested that its reflex arc has either a non-cholinergic synapse or has no synapse at all (5,119). The transmitter at the neuromuscular junction is however cholinergic since atropine prevents these responses (5,126). The relaxation of the longitudinal muscle and the aboral segments of the circular muscle in the emptying phase has been postulated to result from release of an inhibitory transmitter, from some neurone in the intestines. The identity of this transmitter has not yet been established.

(C) The Intrinsic Reflexes of the Small Intestines

Recently Hukuhara et al. (34,35,124,127) studied the intrinsic reflexes in denervated intestinal loops, by stimulation of the muscle layer and the mucous membrane separately. He revealed that two different types of reflexes were evoked, depending on the region stimulated, muscle or mucosa. The "mucosal reflex" elicited by mechanical or chemical stimulation of the mucosa showed excitation orally and inhibition anally, with respect to the site of stimulation. This response was blocked by hexamethonium, cocaine or prolonged anoxaemia and was not affected by section of the extrinsic nerves. It has been suggested that the reflex is identical with the peristaltic reflex (4). The "muscle reflex" was obtained by stimulation of the receptors in the intestinal muscle by application of acetylcholine or Ba etc., on the serosal coat or on isolated flaps of longitudinal muscle. In denervated intestines inhibition was seen on both sides of the stimulated segment. This response could not be easily blocked by hexamethonium or anoxaemia. Investigation of the intrinsic reflexes of the intestines by earlier workers did not yield consistent results. Hukuhara attributes these discrepancies to be due to the mode of stimulation used and the involvement of both reflexes at the same time.

(D) The Intramural Nervous System

The analysis of site of action of excitatory drugs affecting intestinal motility depends on antagonism with specific agents known to act at certain sites, i.e., at the ganglia, nerve endings, neuroeffector sites or on the muscle.

Section of extrinsic nerves, leaves the intrinsic nervous system intact so that complete denervation of the segment is not obtained. Many drugs affecting intestinal motility act wholly or partially on these intrinsic nerves and attempts have been made to damage and/or inactivate them in order to determine the exact site of action of such drugs.

Ganglion-free or plexus-free muscle preparations have been claimed and used to differentiate drug action since Magnus (1904) and Gasser (1924). More recently studies have been made by Evans and Schild (128) and Prosser et al. (90) on such preparations. However it is difficult to assess definitely even microscopically if such a procedure has been successful, or if axons are still present in the muscle. Also it is impossible to say whether the lack of response is due to successful denervation or due to direct damage to the smooth muscle cells and their inability to contract.

Hukuhara et al. (34,129) described a method by which a loop of small or large intestine can be deganglionated and presumably denervated. If time was allowed for axons to degenerate complete denervation was possible. In this method an intestinal loop with its artery and vein was isolated with ligatures tied at either end. Tyrode's solution was perfused through an arterial cannula and all the blood from the segment was expelled, and replaced by the solution. The vessels were clamped and the segment kept ischaemic for 1.5 to 4 hours after which blood supply to the segment was restored. They reported that with 2 hours complete ischaemia, rhythmic contractions reappeared soon after the re-establishment of

blood supply but the intrinsic reflexes particularly the mucosal reflex were more susceptible to oxygen lack and were absent after 15 minutes. They also took longer to recover than the rhythmic contractions. If ischaemia was maintained for >2 hours complete recovery was not observed. The muscle reflex was more resistant and remained intact after ischaemia had been maintained for 1 hour. If a segment was kept ischaemic for >2.5 hours, no recovery was seen after 6 hours. When ischaemia lasted 4 hours in chronic preparations, no recovery was seen after 49 days. Histological examination of sections from these tissues showed cytolysis of the ganglion cells with nuclei crowded to one or other side. This was taken as evidence of damage of the ganglion cells but complete destruction cannot be assured by such studies. Comparison of normal, denervated, and deganglionated loops in the same dog showed that the aganglionic loop was hypertonic relative to the other segments but had a less ability to contract; i.e., with the same basic intraluminal pressure, the amplitude of induced contractions was less. In a more recent paper (88) Hukuhara implied that axons and nerve endings were also absent from aganglionic segments prepared by this method. He based this implication on failure of physostigmine to elicit motility in strips of circular muscle taken from these loops. Contractions occurred only in the strips removed from control segments.

Similar studies were carried out by Szurszewski et al. (130). Constant perfusion of the segment with Tyrode solution was carried out to keep the segment without circulating

blood for 4 hours. Electrical and mechanical activity was studied with electrodes and extraluminal transducers placed in perfused and nonperfused adjacent areas, above and below. His results showed that 1. Slow waves recover 7-10 days after operation. 2. Frequency of the slow waves was reduced. 3. Conduction occurred from the lower control segment. He suggested that although the slow waves are myogenic in origin, the myenteric plexus operates some control mechanism that regulates the slow wave frequency.

Other procedures have been used for inactivation of the intrinsic plexuses. Cooling by reducing bath temperature, supplied with O_2 has been shown to result in loss of reflexes which is reversible. Storage at $4^{\circ}C$ without O_2 is said to permanently damage the nerve structures (5,131,132). Ganglion blocking drugs such as nicotine, hexamethonium, dimethylphenylpiperazine (D.M.P.P.) prevent transmission across synapses in the myenteric and submucous plexuses and are assumed to have little or no direct action on the muscle in doses used to block transmission (5,123,133-136). However the specificity of most of these drugs is uncertain. Puffer poison, tetrodotoxin has been shown to depress nerve function by interfering with the increase in Na conductance in response to depolarisation (13,7,138). Smooth muscles are less dependent on such an increase in Na conductance for depolarisation and tetrodotoxin has little or no effect on smooth muscle activity (75,139). This has also been demonstrated for intestinal muscle (117,140).

It has not been possible to stimulate the nerve axons of the intrinsic plexus selectively by means of drugs nor has it been possible to distinguish drugs acting on axons from those acting proximal to it. A technique of co-axial electrical stimulation was introduced by Paton (141). It consists of passing current pulses from one electrode in the bath fluid in which the intestine is suspended, to another within the lumen. Single stimuli of 50 μ sec duration induced twitches which were reported to be potentiated by eserine. They were insensitive to hexamethonium and nicotine and were abolished by small doses of atropine. From this it was concluded that the structures stimulated were postganglionic cholinergic nerves.

Hartfelder et al. (142) used field stimulation with surface electrodes and 50 cps a.c. current. He reported that with this arrangement hexamethonium reduced the responses to submaximal stimuli and suggested that preganglionic elements were also stimulated.

It was later concluded that pre- as well as post-ganglionic cholinergic fibres were stimulated by transmural stimulation (143).

III. RECEPTORS OF THE SMALL INTESTINES

Though the idea goes back to the time of Ehrlich, the modern concept of pharmacological receptors originated with Langley in 1905. In 1906, Dale (151) was the first to make significant use of it in connection with the sympathetic nervous system. He proposed that epinephrine acted on two types of receptors to give its excitatory and inhibitory effects and used this theory to explain the differential blocking effects of ergot alkaloids.

Ahlquist (144) observed that the existence of an inhibitory and an excitatory receptor was not sufficient to explain all the different effects of sympathomimetic drugs and suggested that the response of a receptor depended on its location.

Using different types of tissues he studied the relative potencies of six racemic sympathomimetic amines most closely related to epinephrine. The drugs studied were:

1. Norepinephrine
2. Methyl-norepinephrine
3. dl-epinephrine
4. l-epinephrine
5. Methyl-epinephrine
6. Isopropyl-norepinephrine

He discovered that the order of relative potency of the drugs differed with different responses. In producing most excitatory responses such as vasoconstriction and uterine contraction and one important inhibitory response, namely

intestinal inhibition, the most potent drug was epinephrine. Norepinephrine was third of the six studied and isopropyl-norepinephrine was the least potent. In producing most inhibitory responses with one excitatory response namely myocardial stimulation, the order of potency was different. Isopropyl-norepinephrine was now the most potent, epinephrine coming next and norepinephrine was the least potent. He therefore classified adrenergic receptors as consisting of two types, alpha and beta. Alpha-receptors were those receptors in which the relative order of responsiveness to three sympathomimetic amines was epinephrine \geq norepinephrine $>$ isopropyl-norepinephrine and the responses of these receptors were blocked by classical adrenergic blocking agents such as phenoxybenzamine and tolazoline. Beta-receptors were those in which the order of relative responsiveness to the amines were isopropyl-norepinephrine $>$ epinephrine $>$ norepinephrine. No blocking agent was then known for these receptors.

Adrenergic drugs produced relaxation of the intestines, depressing both the electrical slow waves and mechanical activity in sufficient doses. Ahlquist classed

the adrenergic receptors in the intestines as alpha and they were the only inhibitory alpha-receptors.

Nickerson and Goodman discovered dibenamine -- a β -haloalkylamine and in discussing its properties as an alpha-adrenergic blocking agent mentioned that dibenamine blocked the pressor responses to epinephrine but did not block the relaxation of the intestines due to the drug (145).

Powell and Slater (146) reported, that a dichloro analogue of isopropyl norepinephrine--Dichloro-isopropyl norepinephrine (DCI), selectively blocked the inhibitory effects of epinephrine and isopropyl norepinephrine. It had no effect on the pressor responses of norepinephrine. Moran and Perkins (147) showed D.C.I. blocked the inotropic and chronotropic effects of epinephrine on the myocardium. This provided another method for distinguishing the alpha and beta-receptors. In addition to the receptors responding in the order of potency first described by Ahlquist, alpha receptors were blocked by dibenamine, dibozane, and tolazoline etc. and beta-receptors were blocked by D.C.I. Extensive reviews on the alpha and beta-adrenergic blocking properties of various groups of drugs has been published (148,149,150,151).

Furchgott (152) discovered that the relaxation of the intestine due to epinephrine was not blocked by either of the alpha-receptor blocking agents or by D.C.I. He concluded that adrenergic receptors in the intestines were different from the alpha and beta-receptors and called them "delta" receptors. In addition he called the receptors responsible for glycogenolysis in the heart "gamma" receptors. These "gamma" receptors were later shown to be blocked by D.C.I. and are likely to be similar to the beta-receptors.

Ahlquist and Levy (58,149,150) reinvestigated the adrenergic receptors in the intact dog ileum and discovered:

1. That phenylephrine, a potent alpha-receptor stimulant caused relaxation of the intestines which was blocked by dibozane.
2. Isopropylnorepinephrine, a beta-receptor stimulant also relaxed the intestines and this response was blocked by D.C.I.
3. The relaxation caused by epinephrine could be blocked only by a combination of D.C.I. and dibozane.

From these results it was concluded that the dog ileum had both alpha and beta-receptors and activation of either one or both produced inhibition.

Biochemistry and Mechanism of Action.

Many attempts have been made to identify and study the nature of the adrenergic receptors. The concept of Drug-Receptor interactions was developed by Clark (1939). Recent advances in this field and the kinetics involved have been discussed in reviews by Ariens (153), Paton (154), Furchgott (155) and MacKay (156).

The biochemistry and structure-activity relationship of adrenergic receptor and the sympathomimetic amines have been studied extensively by Belleau (157) and others. Many have speculated a "substrate-enzyme" relationship between the transmitter and receptor and that adrenergic receptors might be an enzyme possibly monoamine oxidase. This has been excluded by Furchgott et al. (158) with iproniazid and by Belleau and Pindell (159) using norepinephrine labelled with deuterium. Belleau (157) postulates that at the receptor level triggering of the response results from

the electrostatic interaction between the catecholamine and the receptor, and the formation of an ion-pair between the positive nitrogen atom in the catecholamine molecule and a negative anionic site on the receptor. Evidences for this and other alternative theories were discussed.

In spite of the great amount of work done in this field the identification of the adrenergic receptor either biochemically or morphologically has not been accomplished. Recently, Kosterlitz and Watt (160) working on the guinea pig ileum postulated that alpha-receptors are situated in the neurones innervating the longitudinal muscle, and the beta-receptors were in the muscle itself. Rossum and Mujic (161) seems to think that the alpha-receptors of the rabbit jejunum were located on the neurones of parasympathetic ganglion tissue of the intestines. Norberg (16) on the basis of fluoromicroscopic findings had suggested that the effect of adrenergic stimulation was mostly on the postganglionic parasympathetic ganglion cells with very little direct effect. He found adrenergic endings chiefly in the myenteric plexus. Christenson and Daniel (162) working on the oesophagus suggested they were located in the postganglionic cholinergic nerves since the excitatory effects of epinephrine and nor-epinephrine were blocked by tolazoline, hemicholinium and atropine and potentiated by physostigmine. These excitatory effects were not blocked by hexamethonium. Neither were the inhibitory effects of the catecholamines reported by Kosterlitz and Watt (160). All these investigators seem to agree that the beta-receptors were located in the smooth muscle cells.

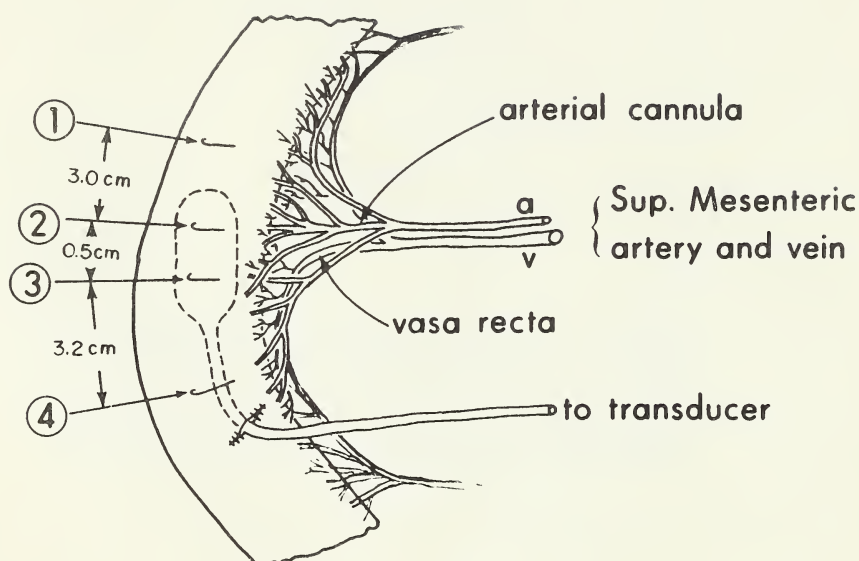
ABBREVIATIONS

i.a.	intra-arterial
i.v.	intravenous
E	epinephrine
NE	norepinephrine
INE	isopropylnorepinephrine
mls	milliliters
DCI	dichloro-isoproterenol
PBZ	phenoxybenzamine
DBZ	dibenamine
P.D.G.	phenyldiguanide
Mch	methacholine
Nict.	nicotine
D.M.P.P.	dimethylphenylpiperizinium
5-HT	5-hydroxytryptamine
NOGFKR	non-oxygenated glucose-free Krebs Ringer solution

SECTION I

EFFECT OF ADRENERGIC DRUGS ON THE ELECTRICAL AND
MECHANICAL ACTIVITY OF THE DOG SMALL INTESTINE

Fig. 1.1.



SEGMENT OF JEJUNUM SHOWING POSITION
OF CANNULA ELECTRODES AND BALLOON

OBJECT OF STUDY

1. To study the adrenergic receptors in smooth muscle of the dog small intestine in vivo by analysing the effects of adrenergic drugs on its electrical and mechanical activity. To avoid homeostatic reflexes and other indirect effects on intestinal activity, drugs were administered intra-arterially.
2. To exclude the possibility that these effects were secondary to ischaemia as a result of vasoconstriction caused by the drug.
3. To determine if cocaine potentiated the effects of catecholamine in the intestines as reported in some tissues in view of the possible lack of direct innervation of smooth muscle by sympathetic nerves.

METHODS

I. THE ELECTRICAL AND MECHANICAL ACTIVITY

The electrical and mechanical activity of the dog small intestines were studied according to the method of Daniel et al. (50).

Male and female dogs 10-15 Kg were used and the area studied was usually the jejunum.

Surgical Procedure

Anaesthesia: The dogs were kept under light anaesthesia throughout the experiment. For induction, a mixture of chloroform 2% and urethane 10% was administered intravenously in a dose of 3 mls/Kg. This was usually sufficient for preliminary surgery, but was supplemented with 30-60 mgm of Nembutal (pentobarbital sodium) when necessary. The dose of barbiturates was kept at a minimum because they often caused a temporary increase in

sympathetic activity which may depress normal spontaneous activity in the intestines. The total experimental period was approximately 6 hours.

Surgery: The dog was placed on its back on a heated operating table and the right femoral vein was prepared for intravenous injections. A tracheotomy was done, and a tracheal tube inserted, and this could be connected to an artificial respirator should this become necessary. The carotid artery and vein were dissected and the artery cannulated with a polythene cannula (size I.D. 0.106"; O.D. 0.138"). This was connected through a T-tube filled with heparinised saline to a Sanborn pressure transducer (Model 467B) and the signals were fed into the input of the L.V.D.T. coupler -- (Type 9805B) and changes in blood pressure were recorded.

The abdomen was opened by a median incision using a cutting cautery. The duodenum was located and traced up gently to the ligament of Trietz. A segment of jejunum 10-20 cms from the ligament of Trietz and with a good collateral blood supply was chosen. The terminal branches of the superior mesenteric artery form arterial arcades in the jejunum from which the vasa recti arise and supply the intestines (14). A vasa recta which perfused an area of 1-2 cms of intestine was carefully selected (Fig. 1.1). The artery was separated from the adjacent vein and nerve and cannulated with a small polythene cannula (size I.D. 0.023"; O.D. 0.038") and intra-arterial perfusions were made through this cannula, at a constant rate in each preparation. A brief infusion of 1-2 mls of heparinised saline was given to delineate the perfused area. With adequate collateral blood supply the restoration of normal colour after stopping the

infusion was rapid. Clotting of blood in the small cannula was prevented with injections of small quantities of heparinised saline (1 unit/ml).

Recording Method: (Electrical Activity) Extracellular recording of the electrical activity was carried out. The different or recording electrode consisted of silver wire 0.006" in diameter and insulated with polythene tubing and cemented except for the last 1 cm or so. This exposed portion was threaded into the intestinal wall just under the serosa where it was in contact with the longitudinal muscle. The position of the electrodes for electrical recording and the balloon for recording mechanical activity is shown in Fig. 1.1.

Two electrodes were placed in the perfused area 0.3 - 0.5 cm apart. One electrode was placed 2 - 3 cms proximal and another was placed 2 - 3 cms distal to the perfused area. The leads were brought out through the abdominal incision and the activity in these electrodes was recorded, a common indifferent electrode being placed in the left thigh. This arrangement caused minimum interference from cardiac electrical activity. The electrical signals were fed into the input of an a-c coupler (type 9806A) and amplifier, with a time constant of 3 secs, and recorded on a six-channel Beckman Offner Type R dynograph. Most recordings were carried out with a sensitivity of 1 mv/cm or 0.5 mv/cm. Frequency of the slow waves was determined by taking the time for 10 slow waves and converting the result into a figure for frequency. The end of the rapid upward (positive) deflection which was usually completed in less than 100 msec. was taken as zero time for all measurements.

The Contractile Activity: This was recorded by means of an inflated condom balloon (2-3 cms long) fitted to a polythene tubing, and placed in the lumen underneath the electrodes (E2 and E3) in the perfused area. The balloon was inserted by a small stab incision made distal to the area under study and stitched in place by sutures at the wound. The position of the balloon was repeatedly checked as it was likely to be pushed distally during peristaltic movements. The balloon was inflated with air, (20-30 mms H_2O) connected to a Sanborn pressure transducer Model 268(B) and the signals were led into the Offner dynograph through the L.V.D.T. coupler -- (Type 9805B) similar to the one used for recording the blood pressure. Changes in the intraluminal pressure were then recorded on one channel of the dynograph. Care was taken to avoid over-distension of the balloon which would stretch the muscle and stimulate contractions. At the same time, too low a pressure may result in total or partial collapse of the balloon during contractions, which will then, not record all of the response.

After the balloon was in place the electrodes were insulated from each other and from the surrounding area by gauze pads dipped in paraffin oil, and the abdominal incision closed with haemostats.

Electrical Activity in the Circular Muscle Layer (3 dogs): A small rectangular flap of longitudinal muscle (1.0 - 2.0 cm) was cut out and separated from the circular layer and reflected. With electrodes placed in the longitudinal and circular muscle layers the electrical activity in the two layers was recorded. In another preparation, the longitudinal muscle layer was stripped off for some distance and electrical activity studied with

electrodes in the circular muscle layer at varying distances from the cut edge of the longitudinal muscle. Electrical activity was also studied after transection of the segment.

II. ADRENERGIC RECEPTORS OF THE SMALL INTESTINES.

Adrenergic receptors in the intestine were investigated according to the methods of Ahlquist et al. (58, 144): 1. By studying the relative potency of epinephrine, (E) norepinephrine (NE) and isopropyl norepinephrine (INE) to produce inhibition of intestinal activity. 2. By specific blocking agents, and observing the change in the order of potency after blockade of one or the other of the receptors.

Response to Drugs and the Relative Order of Potency. (22 dogs)

E, NE and INE were injected into the arterial cannula in doses of 0.1 $\mu\text{g/ml}$, 0.5 $\mu\text{g/ml}$, 1.0 $\mu\text{g/ml}$ and 2.0 $\mu\text{g/ml}$ calculated as their active l-isomers and the effects on the electrical and mechanical activity of the intestines were recorded. The d-isomers are considered inactive. In the following experiments 1.0 $\mu\text{g/ml}$ was used as the most suitable concentration for the study of effects on intestinal activity. These doses produced clear but submaximal responses. E was considered to be most potent on the alpha-receptors with considerable effect on beta-receptors. NE stimulated predominantly the alpha-receptors and INE stimulated predominantly the beta-receptors. Later on phenylephrine was also used to test for any difference between its action and that of NE on intestinal activity and to determine whether or not tolazoline was able to completely block this action. The drugs were always perfused in 1 ml volume of solution and flushed in with 0.5 mls of Krebs Ringer solution. To prevent oxidation of the

TABLE 1.1

ADRENOTROPIC RECEPTORS

(Classification by Ahlquist et al. 1948)

Receptor	Function	Order of Potency Drugs Stimulating	Blocking Agents
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α	Blood vessels (vaso constr)		P.B.Z.
	Uterus	A > NA >> INA	Dibenamine
	Dog		
	Human		
	Intestine		Tolazoline

β	Myocard		D.C.I.
	Blood vessels (vaso dil)	INA > A > NA	Nethalide
	Uterus		Propranolol
	Dog		
	Human		
	Intestine		

catecholamines and the formation of trihydroxyindoles, stock solutions of 50 µg/ml were made up in 0.1 N HCl solution. These were diluted to 10 µg/ml and 1 µg/ml with Krebs Ringer solution. Control perfusion of 2 mls of 0.001 N HCl in Krebs Ringer solution was carried out at various intervals during the experiments, to eliminate the possibility that the observed effects were due to the procedure. Perfusions were made at 5 minute intervals between doses and at 10 minute intervals between series. The relative potency E, NE and INE was estimated by observing the decrease in mechanical activity produced by each dose. This was a more reliable indication of inhibition than decrease in spike activity since a larger proportion of the perfused area was monitored by the balloon than by the electrodes. Since these preparations deteriorated with time, and with repeated drug injections, dose-effect relations had to be based upon relatively few doses. Despite this, using the 1 µg doses, described above, or similar doses, the relative effects and hence the relative potency of the drugs could be effectively studied. The equi-effective doses of the three drugs were also determined.

Effect of Receptor Blocking Agents

The classification of adrenergic receptors by Ahlquist et al. (58, 144), the relative order of potency of E, NE and INE on each type of receptor, and the selective blocking agents are given in Table 1.1.

Adrenergic receptors in the intestines are predominantly alpha. To study the change in the order of relative potency of the catecholamines after blockade of the alpha-receptors, tolazoline 7-10 mgm/Kg was given intravenously to 18 dogs. The effects of E, NE and INE on electrical and mechanical activity

were recorded beginning after 30 minutes and the change in the order of potency was noted. Other alpha-receptor blocking agents such as phenoxybenzamine 10 mg/Kg and dibenamine 25 mg/Kg have also been used in other experiments not included in this series. In another series (3 dogs) the beta-receptors were blocked with propranolol or (AY-64043) in the dose of 3 mgm/Kg given intravenously. The change in the order of potency of E, NE and INE was again noted and their equi-effective doses determined. Another beta-receptor blocking agent Nethalide (or pronethalol) was also used. D.C.I. was not used. The effects of E, NE and INE after both alpha and beta-blocking agents as well as their relative order of potency was studied. The effectiveness of receptor blockade was tested with intravenous injections of NE (1 $\mu\text{g/Kg}$) or INE (3 $\mu\text{g/Kg}$).

Solutions and Drugs

1. Krebs Ringer Solution: Has the following composition. NaCl 115.48 mM., KCl 4.63 mM., CaCl_2 2.47 mM., MgSO_4 1.16 mM., NaHCO_3 21.9 mM., NaH_2PO_4 1.16 mM., Glucose 50 mM., pH 7.4 bubbled with 95% O_2 and 5% CO_2 .
2. Morphine Sulphate: Stock solution of 1 mgm/ml diluted to a final concentration of 100 $\mu\text{g/ml}$.
3. 1-epinephrine Bitartrate: Stock solution of 50 $\mu\text{g/ml}$ calculated as the base in 0.1 N HCl. This was diluted just before use with Krebs Ringer solution to obtain final concentrations of 0.1 $\mu\text{g/ml}$; 0.5 $\mu\text{g/ml}$; and 1.0 $\mu\text{g/ml}$.
4. 1-norepinephrine Bitartrate: Stock solution of 50 $\mu\text{g/ml}$ calculated as the base in 0.1 N HCl.

This was diluted just before use with Krebs Ringer solution to obtain a final concentration of 0.1 $\mu\text{g/ml}$; 0.5 $\mu\text{g/ml}$ and 1.0 $\mu\text{g/ml}$.

5. dl-isopropyl norepinephrine HCl: Stock solution of 50 $\mu\text{g/ml}$ of the l-isomer was dissolved in 0.1 N HCl. This was diluted with Krebs Ringer solution just before use to obtain a final concentration of 0.1 $\mu\text{g/ml}$; 0.5 $\mu\text{g/ml}$ and 1.0 $\mu\text{g/ml}$.
6. Phenylephrine HCl. (Neo-synephrine): Stock solution 50 $\mu\text{g/ml}$. (calculated as the base) in 0.1 N HCl. This was diluted with Krebs Ringer solution to obtain final concentrations of 0.1 $\mu\text{g/ml}$; 0.5 $\mu\text{g/ml}$ and 1.0 $\mu\text{g/ml}$.
7. Tolazoline: 25 mg/ml solution given in a dose of 7-10 mgm/Kg.
8. Phenoxybenzamine: Calculated as the salt; 10 mgm/Kg dissolved in 10 mls warm propylene glycol. Diluted with 20 mls Krebs Ringer solution just before infusion. This was used as an alternative to tolazoline as an alpha-blocking agent.
9. Dibenamine: Calculated as the salt, 25 mgm/Kg dissolved in the same way as phenoxybenzamine. This was also used as an alternative for tolazoline.
10. Propranolol (AY-64043, Ayerst, McKenna and Harrison Ltd., Montreal): Given in a dose of 3 mgm/Kg in 10 ccs Krebs Ringer solution.
11. Nethalide (Pronethalol): 3 mgm/Kg in 10 ccs Krebs Ringer solution.

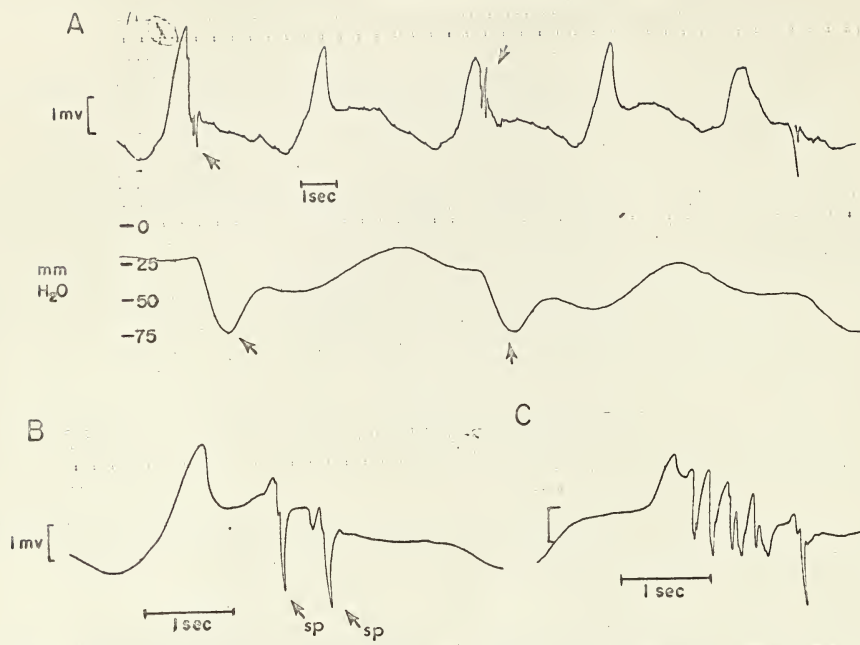


Fig. 1.2.

- A) Spontaneous activity in the dog jejunum showing slow waves, spikes and contractions. Arrows show the correlation between appearance of spikes and contractions.
- B) Components of the electrical potential. The slow positive deflection and fast negative spikes (SP) on the plateau.
- C) Increase in number of spikes after morphine 200 μg/kg I.V.

RESULTS

I. THE ELECTRICAL AND MECHANICAL ACTIVITY OF THE SMALL INTESTINE.

After opening the abdomen and completion of surgery, due to reflex sympathetic inhibition, the intestines were usually quiescent. The electrical record of such inactive segments showed only slow waves though some activity may be seen in the mechanical record possibly because the balloon was recording changes in intraluminal pressure from a much larger area. Activity usually resumed about 30 minutes after surgery.

Configuration of the Slow Wave: The slow wave in the duodenum and jejunum consisted of a sharp positive deflection (2-4 mv) followed by a smaller negative deflection and a plateau. When spontaneous activity was present a variable number of fast spikes appeared superimposed on the plateau of the slow wave and was accompanied by contractions in the mechanical records Fig. 1.2 (A). There was a definite relationship between the appearance of spikes and of contractions, the spikes preceding the contractions by 100-500 msec. In the ileum the slow waves were smaller with a slower rate of rise and an equally slow return to the original potential. Fig. 1.2 (B) shows the various parts of a typical slow wave from the jejunum. Fast spikes are shown by arrows (SP). When activity was induced by intravenous injection of morphine 100-200 µg/ml or physostigmine 100 µg/ml the amplitude of the slow waves usually became greater and there was a marked increase in the number of spikes Fig. 1.2 (C). There was also a parallel increase in height of contractions in the mechanical record. The effect of morphine lasted for several hours and provided

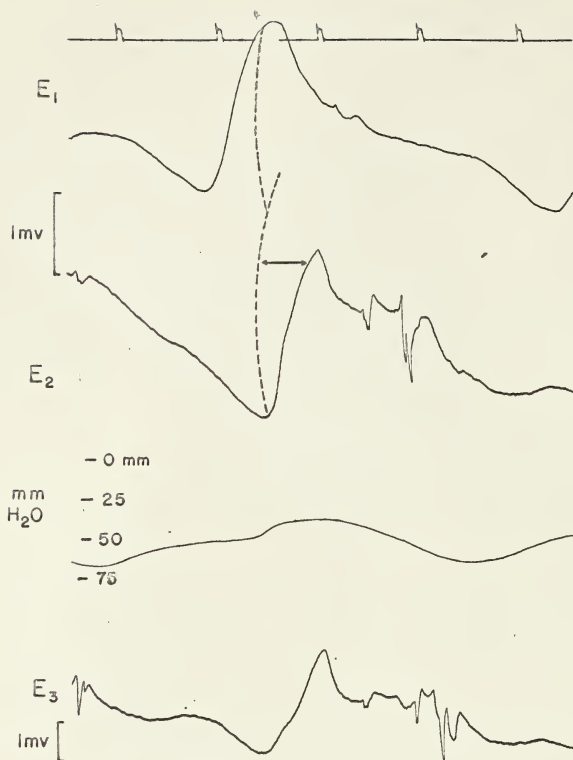


Fig. 1.3. Conduction of slow waves in the jejunum.
 E_1 is oral to the others.

Distance between $E_1 - E_2 = 3.2$ cm.

$E_2 - E_3 = 0.5$ cm.

The displacement of the slow wave shown by the dotted line and the arrow indicate the time (0.5 sec) taken by the slow wave to travel the distance between $E_1 + E_2$ (3.2 cm). Markings at the top indicates 1 sec.

a suitable active preparation for the study of intestinal inhibition by adrenergic drugs.

Frequency of the Slow Waves. The frequency of slow waves varied in different parts of the intestines. In the duodenum and jejunum the usual rate recorded was roughly 16-20/min. and in the ileum 13-15/min. slowing to 7-10/min. in the terminal ileum. These values agreed well with those obtained by other investigators (33, 50, 55). The frequency was found to vary with temperature and the period during the experiment, at which it was studied. In the latter part of the experiment possibly due to the slow deterioration of the muscle the rate of the slow waves was found to slowly decline.

Propagation of Slow Waves. Slow waves in the jejunum were found to be propagated for varying distances. Propagation of spikes on the other hand was usually not observed if the electrodes were much further than 1.0 cm apart. Fig. 1.3 shows propagation of slow waves in the jejunum. E1 was oral to E2 and E3 and the mechanical record is shown between E2 and E3. The distance between E1 and E2 is 3.2 cm and the time taken for the wave to travel this distance was approximately 0.5 secs. This is shown by the dotted line and the displacement of the peak of the slow wave (arrow) which represents the time taken by the slow wave to travel the distance between E1 and E2 (3.2 cms). The conduction velocity in this particular segment was therefore 6.4 cm/sec. The value for this region given in literature was 6-10 cm/sec (4, 50).

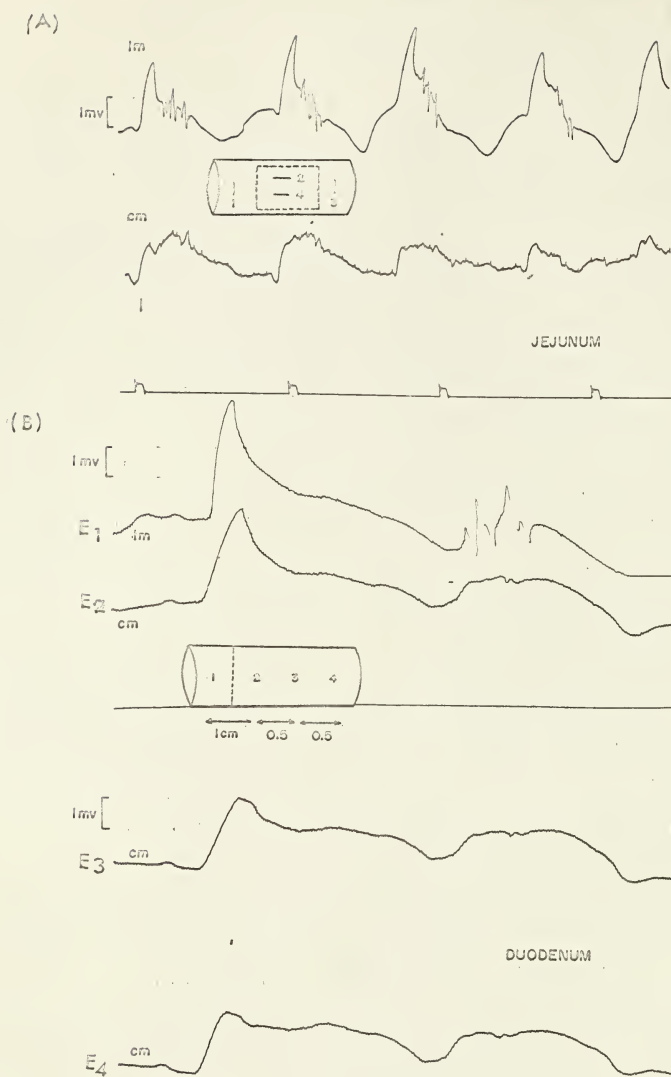


Fig. 1.4. Slow waves and spikes in the longitudinal and circular muscle.

- (A) Jejunum - Dotted area shows the area of circular muscle with the longitudinal flap removed. Electrodes (numbered) placed as shown.
- (B) Duodenum - Electrode 1 in the longitudinal muscle 2, 3, 4 in the circular muscle with the longitudinal muscle stripped off and the distances as marked. lm = longitudinal muscle cm = circular muscle.

Electrical Activity in the Circular Muscle (3 dogs).

When a flap of longitudinal muscle had been cut away and two electrodes were placed in the circular muscle and two in the longitudinal muscle on either side (Fig. 1.4 (A)) the slow waves in the longitudinal muscle showed the characteristic wave form described previously. They were of normal amplitude and had a varying number of fast spikes. The slow waves recorded from the circular muscle from which the flap of longitudinal muscle had been cut away were smaller, distorted and had small indefinite spikes. Fig. 1.4 (A). In another preparation the longitudinal muscle was totally cut away except for a small strip in the mesenteric border at which the blood vessels entered. Electrode E1 was placed in the longitudinal muscle and E2 to E4 were placed in the circular muscle at varying distances from the cut edge. Slow waves were recorded from E1 in the longitudinal muscle and E2 in the circular layer, 0.5 cm away from the cut edge. The slow waves had the same configuration and were roughly the same size Fig. 1.4 (B). No spikes were seen in E2. In E3 and E4 1.0 cm and 1.5 cm further away, the amplitude of the slow waves was reduced 50% and the rate of rise and fall was slower. In a third preparation when the longitudinal muscle was entirely stripped away and the segment transected, no definite slow waves were seen, and only small, irregular and undefined waves were recorded.

II. ADRENERGIC RECEPTORS OF THE SMALL INTESTINE

To observe the inhibitory effects of adrenergic drugs better, activity had been induced by an i.v. administration of morphine 100-200 $\mu\text{g/Kg}$ 15-30 minutes before recording.

(The action of morphine on the intestine will be discussed later). To eliminate the possibility that morphine could interfere with the interpretation of the results, control experiments were carried out in which the relative potency of epinephrine, (E) norepinephrine (NE) and isopropyl norepinephrine (INE) before and after morphine 100-200 $\mu\text{g}/\text{Kg}$ i.v. was studied. Except for the fact that the changes were more apparent because of increased basic activity no significant difference in the records was seen at least in the order of potency and with these drugs.

II. ADRENERGIC RECEPTORS OF THE SMALL INTESTINE.

Relative Order of Potency of E, NE, and INE on Intestinal Activity.

When the activity of the intestines had settled down to a constant basal pattern, i.e. perfusions of E, NE and INE were commenced. The control perfusion of 2 mls of 0.001 N HCl produced little or no change in activity of the intestines (see Methods). 0.1 $\mu\text{g}/\text{ml}$ and 0.5 $\mu\text{g}/\text{ml}$ of all three adrenergic drugs usually abolished the spikes in the electrical record, and reduced the height of contractions in the mechanical record. At such low doses the slow waves were usually unaffected. At a higher concentration of 1-2 $\mu\text{g}/\text{ml}$, slow waves were also affected. The dose required to produce a detectable response differed with the sensitivity of the animal, the area of perfusion and the efficiency of the collateral blood supply in the removal of the drug from the area. Small doses produced inconsistent results and doses larger than 3 $\mu\text{g}/\text{ml}$ caused vasoconstriction and prolonged blanching of the area. 1.0 $\mu\text{g}/\text{ml}$ was therefore chosen as the suitable dose for study of the intestinal activity.

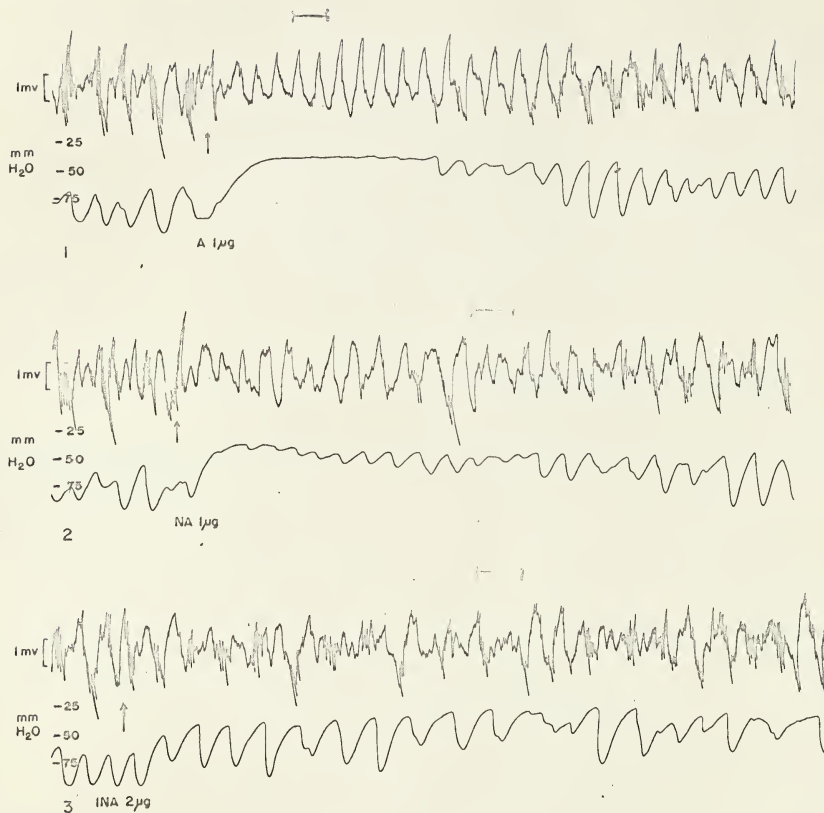


Fig. 1.5. Electrical activity of the jejunum with 1 μ g E and NE ia and 2 μ g INE ia showing the disappearance of spikes in the electrical record and contractions in the mechanical record. Drugs were given at the arrows. Order of potency A > INA > INA x 2. Horizontal line = 4 sec

When no response was seen, the dose was increased. Epinephrine or norepinephrine 1.0 $\mu\text{g}/\text{ml}$ decreased intestinal tone, (indicated by the lowered base line in the mechanical record) and abolished or markedly reduced contractions for a variable period. There was a concomitant reduction or absence of spikes in the electrical activity recorded by E2 and E3 in the perfused area. The amplitude of the slow waves was usually reduced by this dose of E or NE but sometimes, the absence of spikes was the only effect seen in the electrical record. An increase in frequency may or may not be present. Little or no change was observed in E1 and E4, outside the perfused area.

INE was usually without effect in this dose but some effect could be observed when the dose was raised to 2 μg .
Fig. 1.5. In the antrum it has been reported that the ratio of equi-effective doses of NE and INE varied from 1:50 to 1:4 (163). An excitatory response to higher doses of INE was seen in five out of the twenty-two animals studied. E and NE caused inhibition in these preparations. In these cases usually no response was seen to 1 μg and 2 μg of INE. The increase of dose to 3 μg and 4 μg induced fast spikes and contractions Fig. 1.6. Such action has also been reported by Ahlquist et al. (151) in ileum and by Daniel (163) in the duodenal bulb where the incidence was seven out of ten experiments, and where it occurred in preparations where balloons had not been used.

The order of potency of the catecholamines in producing intestinal inhibition was $E > NE \gg INE$ (19 dogs) or $NE > E \gg INE$ (3 dogs). The former was the order of responsiveness



Fig. 1.6. Stimulation of electrical and mechanical activity in the jejunum with higher doses of INE. (Incidence 1 in 7). Drugs were given at the arrow. Increase number of spikes and a rise in tension is shown. Horizontal line = 4 secs

shown by alpha-adrenergic receptors and indicated their predominance in the intestines. Stimulation of these receptors produced inhibition.

Effect of I.V. Injection, of NE and INE on Blood Pressure and Intestinal Activity.

In order to test for the effectiveness of receptor blocking agents later on, i.v. injections of NE and INE were administered as controls. Intravenous NE 1 $\mu\text{g}/\text{Kg}$ produced a rise in blood pressure of 40-60 mmHg. with little or no change in the electrical and mechanical activity of the intestines. NE 3-5 $\mu\text{g}/\text{Kg}$ i.v. produced a rise in blood pressure of 80-100 mmHg. and a transient inhibition of the electrical and mechanical activity in all the four electrodes Fig. 1.7 (A). Intravenous injection of 1-3 $\mu\text{g}/\text{Kg}$ INE produced a fall in blood pressure of 50-60 mmHg. and little or no effect on the electrical record Fig. 1.8 (A). A transient inhibition of activity was sometimes seen in the mechanical record.

Responses to NE After Alpha-Receptor Block.

Tolazoline 7-10 mg/Kg given i.v. usually blocked partially the intestinal responses to i.a. perfusions of NE Fig. 1.9. This blockade could however be overcome by increasing the dose of the drug e.g., to 2 μg . Fig. 1.10. Blood pressure responses and intestinal responses to i.v. injection of NE were more effectively blocked than the intestinal responses to i.a. perfusion. Norepinephrine i.v. after tolazoline 10 mg/Kg produced a fall in blood pressure similar to the response obtained with E after alpha-receptor block Fig. 1.7 (B). Intestinal responses to NE were never totally

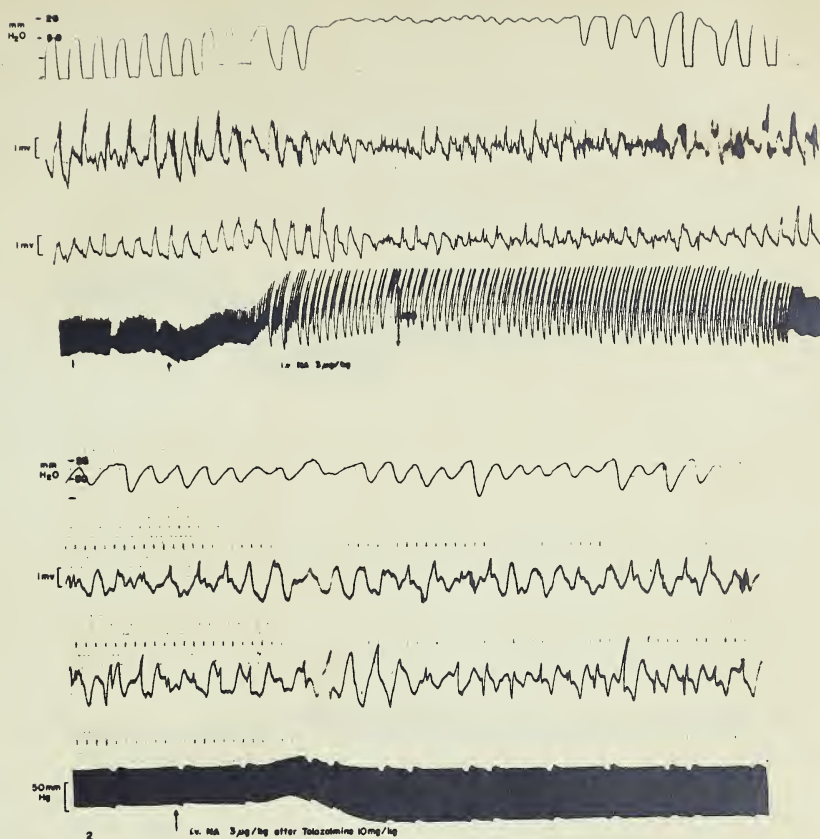


Fig. 1.7. Effect of I.V. NE 3 µg/kg on blood pressure and the electrical and mechanical activity of the jejunum before and after Tolazoline 10 mg/kg I.V. Small effect on the electrical activity and a greater effect on blood pressure and mechanical activity. Blockade of effects on the intestines and reversal of the blood pressure response seen in (B).

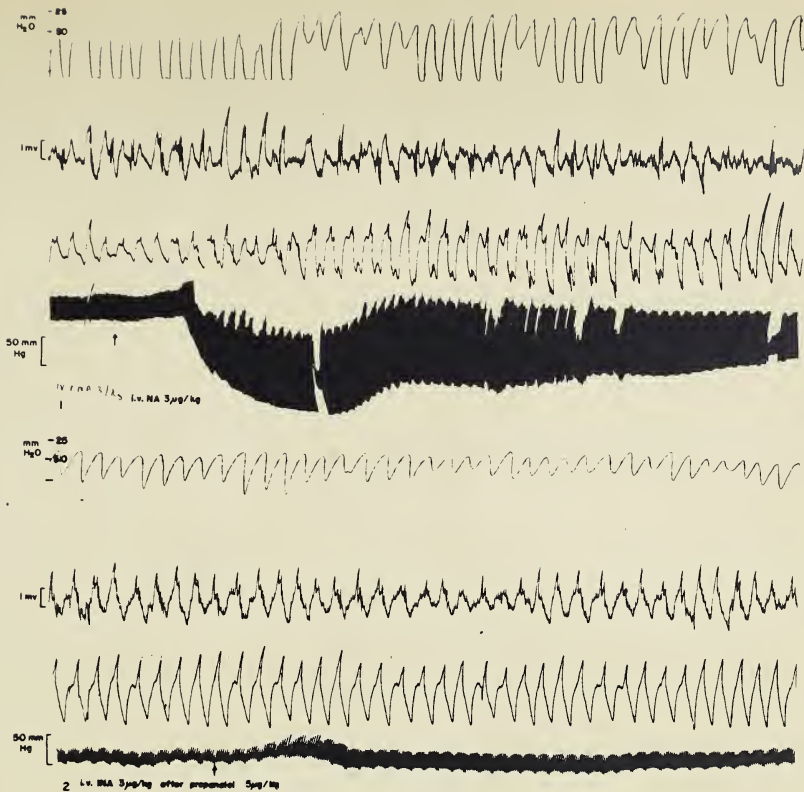


Fig. 1.8. Effect of I.V. INE 3 μ g/kg on blood pressure and electrical and mechanical activity of the jejunum before and after propranolol 3 mg/kg.- Very little effect on intestinal activity. Reversal of blood pressure response to excitation seen in (B).

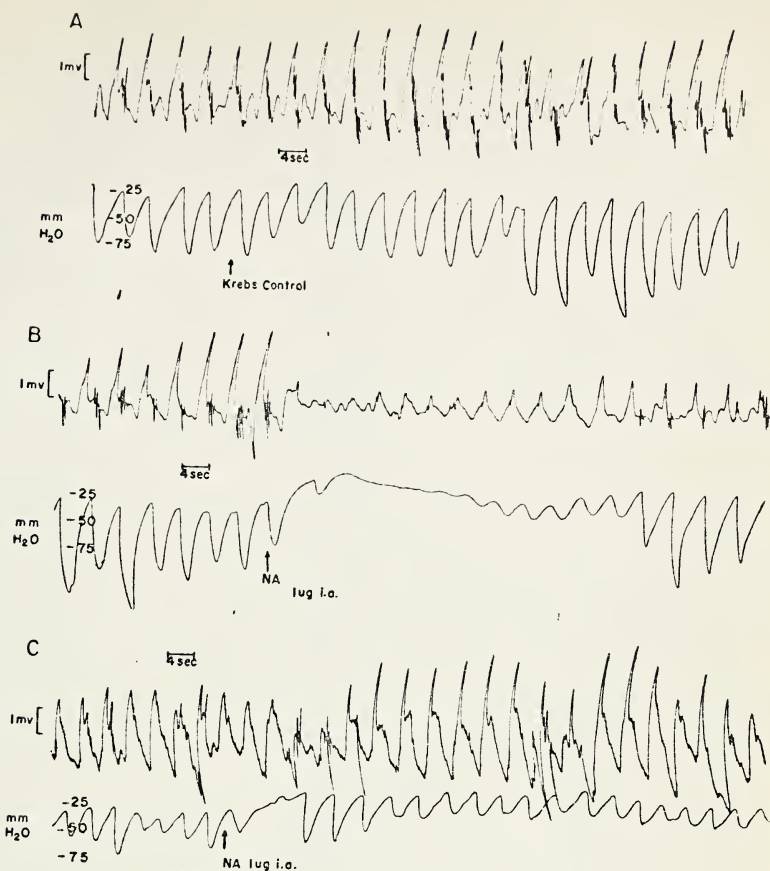


Fig. 1.9. Effects of (A) perfusion with Krebs Ringer solution. No change in electrical and mechanical activity. (B) i.a. NE 1.0 μ g before α Block. Disappearance of spikes and contractions and reduction in the amplitude of the slow waves. (C) i.a. NE 1.0 μ g after Tolazoline 10 mgm/kg showing a nearly complete block.

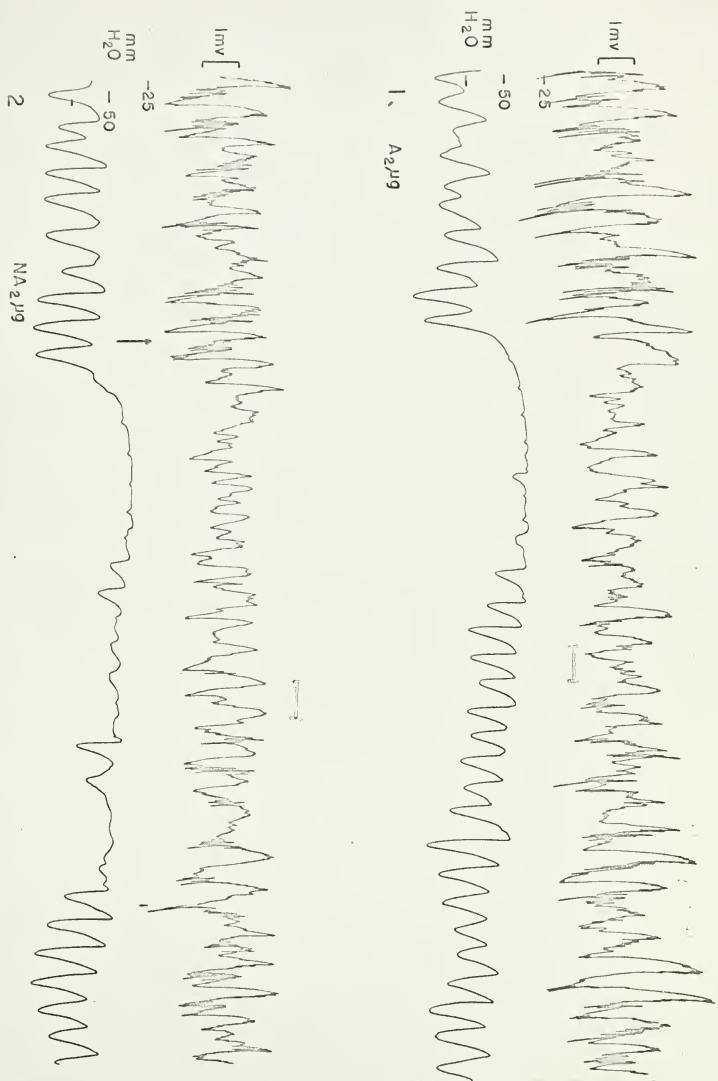


Fig. 1.10. Electrical and mechanical activity of the jejunum of the dog. Inhibition with i.a. injections when A and NA dose raised to 2 μg after α Block. Order of potency in the control was A ≥ NA > INA. No inhibition was seen with NA 1 μg after α Block. Horizontal line = 1 sec.

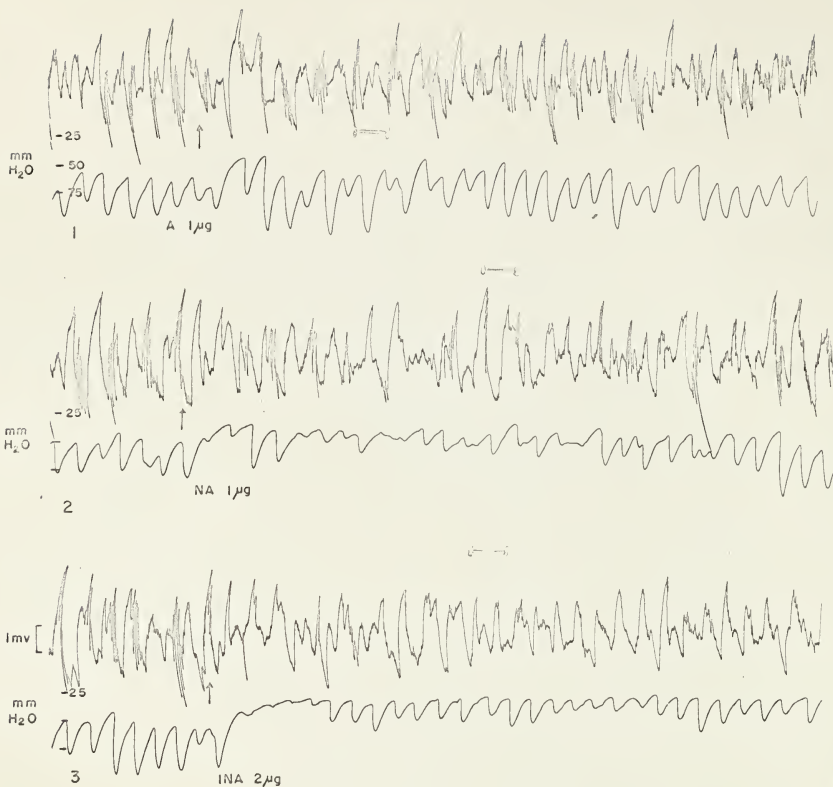


Fig. 1.11. Electrical activity of the jejunum with 1 μ g E and NE i.a. and 2 μ g INE i.a. after α receptor block with Tolazoline 10 mgm/kg. INE 2 μ g is now the most potent. Drugs given at the arrows.

Horizontal Line = 4 sec

abolished even after raising the dose of tolazoline to 15 mg/Kg. The effect of 4 µg phenylephrine, or more, which was approximately equivalent to 1 µg of E or NE was totally prevented by tolazoline. This amine has been reported to have little effect on the beta-receptors.

Blocking agents other than tolazoline were also used in earlier experiments. Phenoxybenzamine 10 mg/Kg or dibenamine 25 mg/Kg produced a rapid and profound fall in blood pressure, in spite of the very slow infusion given (1-2 mls/min.), resulting in a deleterious condition of the segment and poor electrical activity. Phenoxybenzamine and dibenamine also exhibit anti-cholinergic, antihistamine and antiserotonin effects. Therefore the use of these two drugs was abandoned.

Relative Order of Potency of E, NE and INE after Alpha-Receptor Block.

Inhibitory effects of E and INE were never completely blocked by any of the alpha-receptor blocking agents used. The blocking agents were more effective at blocking the responses to NE and E and they had no effect on the responses to INE. Fig. 1.11. The relative order potency of E, NE and INE after tolazoline 10 mgm/Kg was $INA (2 \mu g) \gg E > 1 \mu g \gg NE (1 \mu g)$ (Before tolazoline, the effect of INE 2 µg was much less than 1 µg of NE or E.) This was the relative order of responsiveness of

TABLE 1.2

RESULTS OF EXPERIMENTS OF 22 ♂ AND ♀ DOGS 10-15 Kg.

No. of Experiments	CONTROLS	After Receptor Block (Tolazoline 7-10 mg/Kg I.V.)
11	A ≥ NA >> INA 1 μg ≥ 1 μg >> 2 μg	INA > A ≥ NA 2 μg ≥ 3 μg ≥ 3 μg
3	NA > A >> INA	
5	A ≥ NA >> INA 1 μg ≥ 1 μg >> 1 μg 2 μg INA produced ↑	INA > A > NA
	CONTROL	After β Receptor Block (Propranolol 3 mg/Kg I.V.)
3	A ≥ NA >> INA 1 μg ≥ 1 μg ≥ 2 μg	NA > A > INA 1 μg ≥ 1 μg ≥ 4 μg
	CONTROL	After both α and β Block
2	A ≥ NA >> INA	NA = A > INA 2 μg = 2 μg = 4 μg

1

2

3

4

5

beta-receptors to these amines. These observations thus confirmed the findings of Ahlquist et al. (58, 144) that the dog intestine had both the alpha and beta-receptors, both of which produced inhibition. The findings also suggested an action of each of the drugs on both receptors, predominantly on one or the other receptor.

Relative Order of Potency After Beta-Receptor Block.

Propranolol 3 mg/Kg was given i.v. to block the beta-receptors of the intestine (3 experiments). The response to INE was nearly blocked, but complete prevention of the response was not obtained. Since the alpha-receptors were predominant in the intestines, the relative order of potency of E, NE and INE remained unchanged when the beta-receptor antagonist was present. NA was then more potent than E in producing intestinal inhibition and the dose of INE had to be doubled to produce the same effect. After beta-receptor blockade the following relative order of effectiveness applied was NA (1 μ g) E (1 μ g) INE (4 μ g).

Blockade of Both Alpha and Beta-Receptors: This was attempted many times but the mortality rate of the animals was high, the dogs dying of severe cardiovascular collapse before completion of the experiment. In two preparations, blood volume was maintained with whole blood transfusion and both the blocking agents were given, propranolol first followed by tolazoline. There was a reduction in the response to all the three drugs and the order of potency was the same as it was originally. A summary of results on 22 experiments is presented in Table 1.2.

III. EFFECT OF ADRENERGIC DRUGS ON INTESTINAL BLOOD FLOW.

These experiments were designed to eliminate the possibility that the observed effects were the result of ischaemia due to vasoconstriction produced by the drugs.

METHODS

Anaesthesia: The same urethane-chloralose mixture with small doses of pentobarbital sodium was used to keep the dog under light anaesthesia.

Surgery: The surgical procedure carried out was similar to that described in previous methods. For blood flow studies a branch of an artery further up the arterial arcade was selected and cannulated close to the junction with the main branch, and directed towards it, so that drugs may be flushed in with the normal blood flow. The vein draining the segment was also cannulated (size I.D. 0.070"; O.D. 0.110") and the cannula connected to a dropper. The segment and mesentery were sectioned and isolated and the blood drops from the venous cannula were recorded by a photoelectric cell and monitored in one channel of the dynograph. Simultaneous recording of the blood flow and the electrical and mechanical activity was carried out. The blood drops were collected in a beaker with a small amount of heparinised saline, transferred to an infusion bottle and the blood was returned to the animal through a cannula in the femoral vein. To prevent blood clotting in the small cannulae and during transfusion, 3 mg/Kg of heparin was given to the animal after completion of surgery and repeated in 3-4 hours.

Drug Solutions Used.

1. Epinephrine Bitartrate: Stock solutions of 50 $\mu\text{g/ml}$ were prepared in 0.1 N HCl. Final dilutions with Krebs Ringer solution to obtain a final concentration of 0.03 $\mu\text{g/ml}$; 0.2 $\mu\text{g/ml}$ and 0.3 $\mu\text{g/ml}$.
2. Norepinephrine Bitartrate: Stock solution of 50 $\mu\text{g/ml}$ in 0.1 N HCl. Final dilutions with Krebs Ringer solution to 0.03 $\mu\text{g/ml}$; 0.2 $\mu\text{g/ml}$ and 0.3 $\mu\text{g/ml}$.
3. Isopropylnorepinephrine HCl: Stock solution of 50 $\mu\text{g/ml}$ in 0.1 N HCl. Final dilution with Krebs Ringer solution to 0.03 $\mu\text{g/ml}$; 0.2 $\mu\text{g/ml}$ and 0.3 $\mu\text{g/ml}$.
4. Pituitrin: 5 units/ml.

Drugs were injected into the arterial cannula as in the previous series. 1 ml of drug solution was always used and flushed in with 0.5 mls of Krebs Ringer solution. The relation in time between the onset of drug effects on electrical and mechanical activity and the onset of changes in blood flow was determined.

Pituitrin was expected to produce vasoconstriction by acting directly on the muscle but not on adrenergic receptors. Its action on intestinal activity and blood flow was also recorded and the time relation observed and compared with the catecholamines.

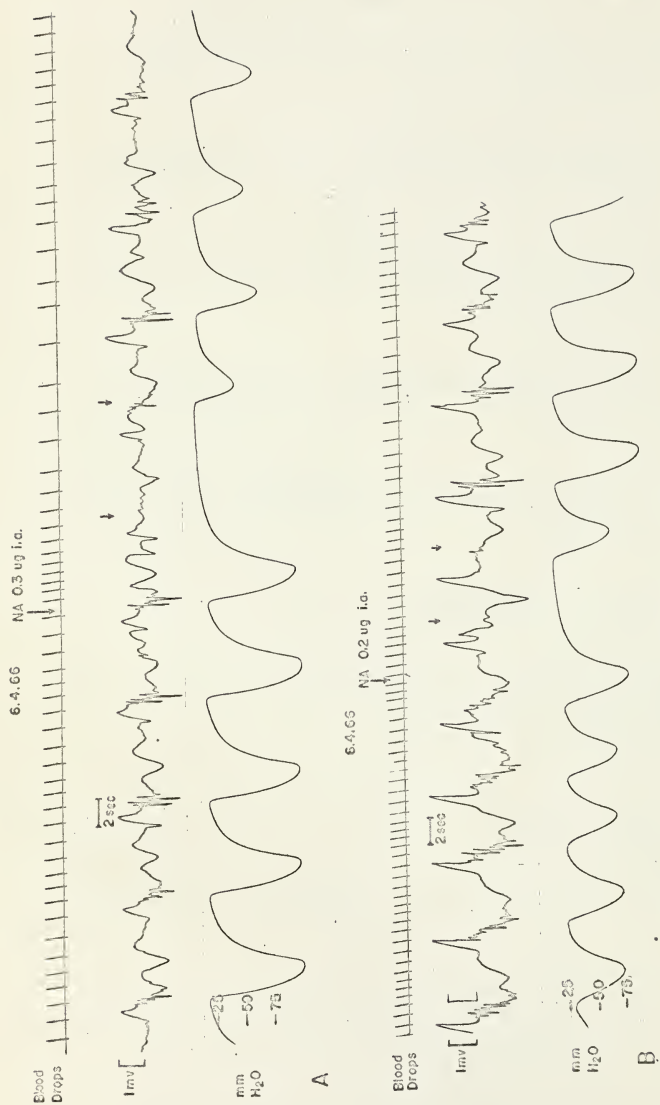


Fig. 1.12. Blood flow studies of the jejunum.

- A) Relation between inhibition of electrical and mechanical activity by NA and reduction in blood flow. First arrow shows absence of fast spikes before decrease in blood flow. Second arrow shows the reappearance of spikes during maximum decrease.
- B) Intestinal relaxation and disappearance of spikes (at two arrows) without significant decrease in blood flow. Blood drops recorded with a photo electric cell.

RESULTS

CHANGES IN INTESTINAL ACTIVITY AND BLOOD FLOW DUE TO CATECHOLAMINES.

Epinephrine, norepinephrine and isopropyl norepinephrine on the isolated segment produced inhibition of intestinal activity even in low doses ($0.03 \mu\text{g} - 0.3 \mu\text{g}$). E and INE did not produce a reduction in blood flow but injection of NE was usually followed approximately 15 secs. later by a slowing of the rate of recorded blood drops. Records of the time of onset of the effects of NE on intestinal activity and the time of onset of the effects of blood flow showed that the reduction in blood flow did not cause the inhibition of activity. In the electrical records i.a. norepinephrine $0.2 \mu\text{g}$ and $0.3 \mu\text{g}$ caused fast spikes and contractions to disappear in the cycle immediately following the injection (2-4 secs.) and before changes in blood flow was detected. Reduction in rate of formation of the blood drops occurred 12-15 secs. later when the intestinal activity had started to recover. Fig. 1.12 (A and B) shows this effect. Spikes and contractions disappeared at the first arrow in both records, when blood flow had not yet been reduced. The first spike reappeared at the second arrow Fig. 1.12 (A), which was the time of maximum decrease in blood flow. In other words, the effects on intestinal activity did not follow the reduction in blood flow due to vasoconstriction. In other instances there may be changes in blood flow with no effect on the electrical and mechanical activity Fig. 1.13 and vice versa. Administration of larger doses of NE ($\geq 1 \mu\text{g}$) produced a reduction in blood flow unrelated to the duration of inhibition of activity.

Pituitrin 5 units i.a. produced inhibition of intestinal activity at approximately the same period at which there was maximum reduction in blood flow (15-20 secs.).

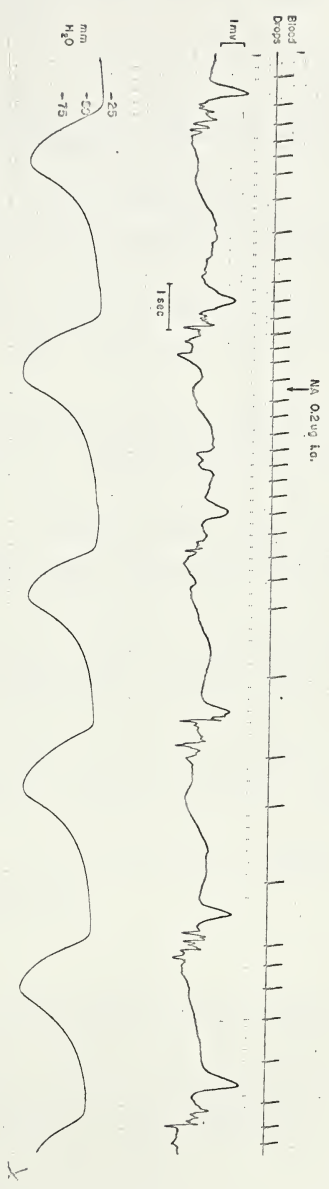


Fig. 1.13. Blood flow studies of the jejunum. NA producing reduction in blood flow with very little change in electrical and mechanical activity.

Intestinal inhibition due to pituitrin may partly be the result of ischaemia. Both the intestinal activity and the blood flow recovered at approximately the same time.

IV. POTENTIATION BY COCAINE.

METHODS

Surgery and Experimental Procedure: These were similar to the procedures described in the preceding "Methods".

Drug Perfusions: Threshold and subthreshold doses of E and NE were used in order that the potentiating effects of cocaine could be better observed. The least effective dose was usually in the range of 0.03 - 0.3 $\mu\text{g/ml}$ for i.a. perfusions and 1 $\mu\text{g/Kg}$ was used for i.v. injections. Intra-arterial perfusions of E and NE were administered at 5 minute intervals in doses ranging from 0.03 μg - 0.3 μg . The least effective dose and two other doses above this were used for study of the potentiation effect of cocaine. Cocaine 2.5 mgm/Kg and 5 mgm/Kg were given intravenously and 15-30 minutes later i.a. perfusions of the catecholamines were repeated. Slow and fast perfusions were carried out to determine if the rate of perfusion made a significant difference in the results obtained.

Drug Solutions Used.

1. Epinephrine Bitartrate: Stock solution 50 $\mu\text{g/ml}$ in 0.1 N HCl. Final concentration in Krebs Ringer solution of 0.03 $\mu\text{g/ml}$; 0.05 $\mu\text{g/ml}$; 0.1 $\mu\text{g/ml}$ and 0.3 $\mu\text{g/ml}$.
2. Norepinephrine Bitartrate: Stock solution 50 $\mu\text{g/ml}$ in 0.1 N HCl. Final concentrations of 0.03 $\mu\text{g/ml}$; 0.05 $\mu\text{g/ml}$; 0.1 $\mu\text{g/ml}$ and 0.3 $\mu\text{g/ml}$.

3. Isopropyl norepinephrine HCl: Stock solution
50 $\mu\text{g/ml}$ in 0.1 N HCl. Final concentrations
of 0.03 $\mu\text{g/ml}$; 0.05 $\mu\text{g/ml}$; 0.1 $\mu\text{g/ml}$ and 0.3 $\mu\text{g/ml}$.
4. Cocaine: 2.5 $\mu\text{g/ml}$ and 5.0 $\mu\text{g/ml}$.

RESULTS (6 dogs)

Epinephrine and norepinephrine 0.1 μg and 0.2 μg i.a. produced transient relaxation and cessation of contractions in the mechanical record and abolished spikes in the electrical record. Very little other effect was seen in the electrical records at this dose.

Cocaine 2.5 mg/Kg i.v. did not potentiate the effects of i.a. perfusions of NE, Fig. 1.14 A or E, Fig. 1.14 B when the perfusions were given at the usual rate. With slower rate of perfusion no significant difference was seen in the magnitude of the inhibition but the duration was slightly prolonged (3 preparations). Cocaine 2.5 mg/Kg i.v. however potentiated the pressor effects of intravenous injection of NE in all the six preparations and the intestinal effects in four preparations Fig. 1.15 (A and B). In two others, NE (1 $\mu\text{g/Kg}$) produced no intestinal inhibition before or after cocaine. Effects of INE were not potentiated either in i.a. perfusions or i.v. injections.

In higher doses (5 mg/Kg) cocaine inhibited the intestinal inhibition produced by all catecholamines in each of three experiments Fig. 1.16 (A and B). However, intravenous NE was still potentiated.

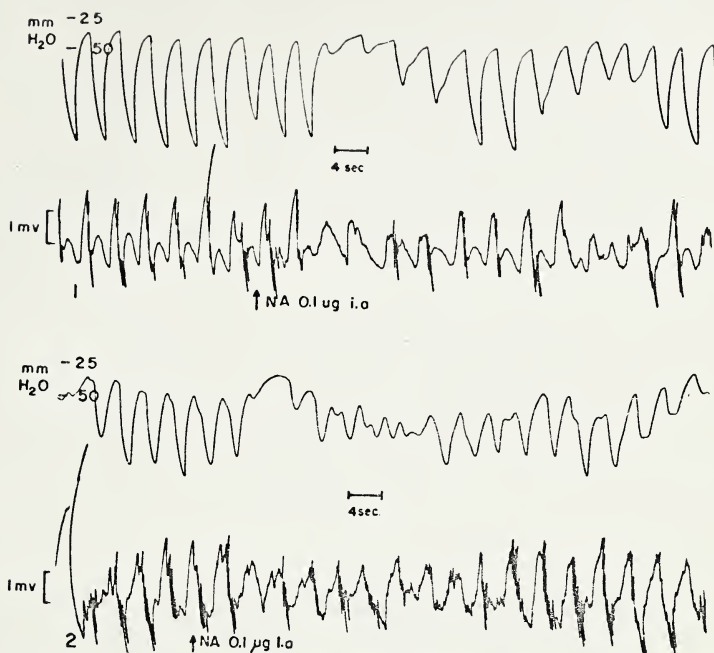


Fig. 1.14 (A) Effect of i.v. injection of Cocaine 2.5 mg/kg on i.a. perfusion of NA 0.1 μ g (1) before (2) after Cocaine. Horizontal Line = 4 sec.

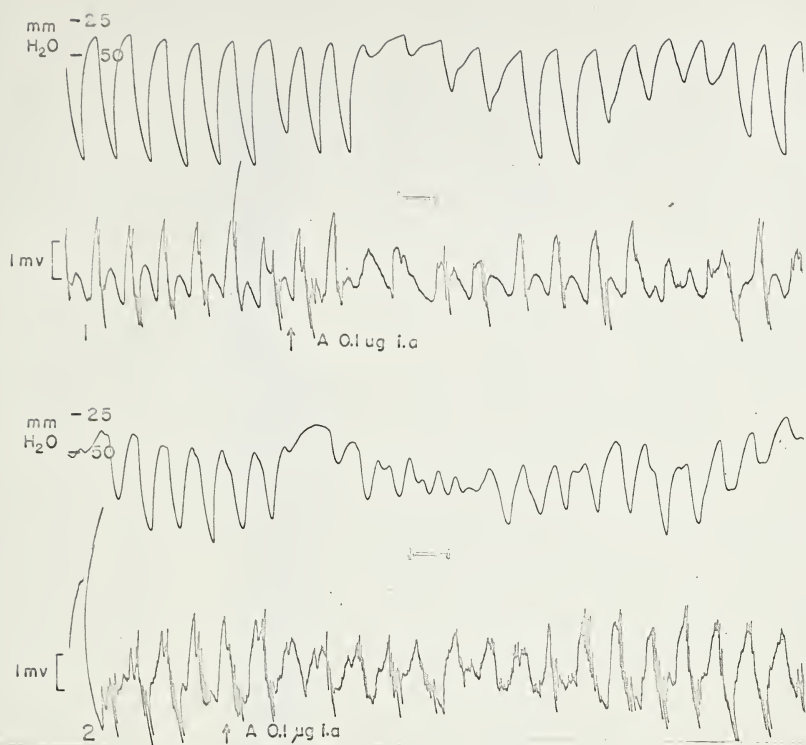


Fig. 1.14(B) Effect of i.v. injection of Cocaine 2.5 mg/kg on a.a. perfusions of Adrenaline 0.1 μ g (1) before (2) after Cocaine. Horizontal Line = 4 secs.

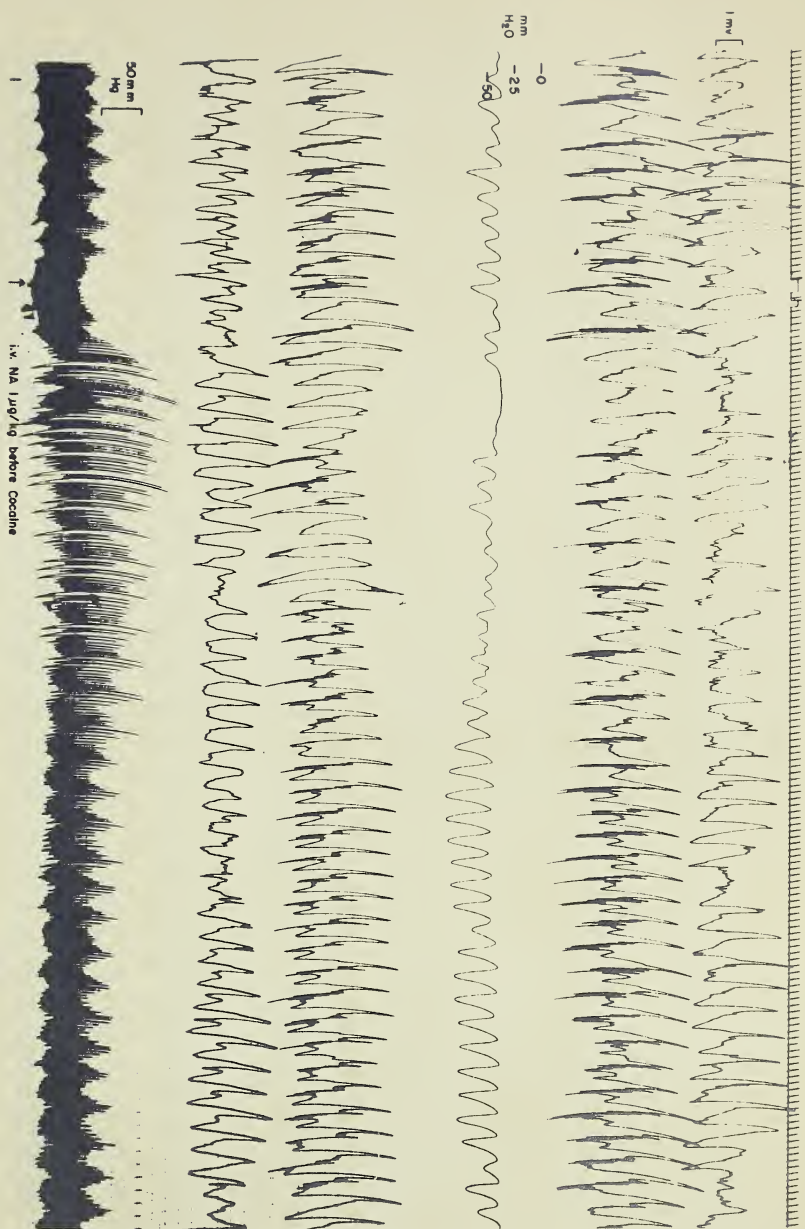


Fig. 1.15 (A). Effects of i.v. NE 1 µg/kg before Cocaine. Effects more pronounced on blood pressure and mechanical activity than on electrical activity.

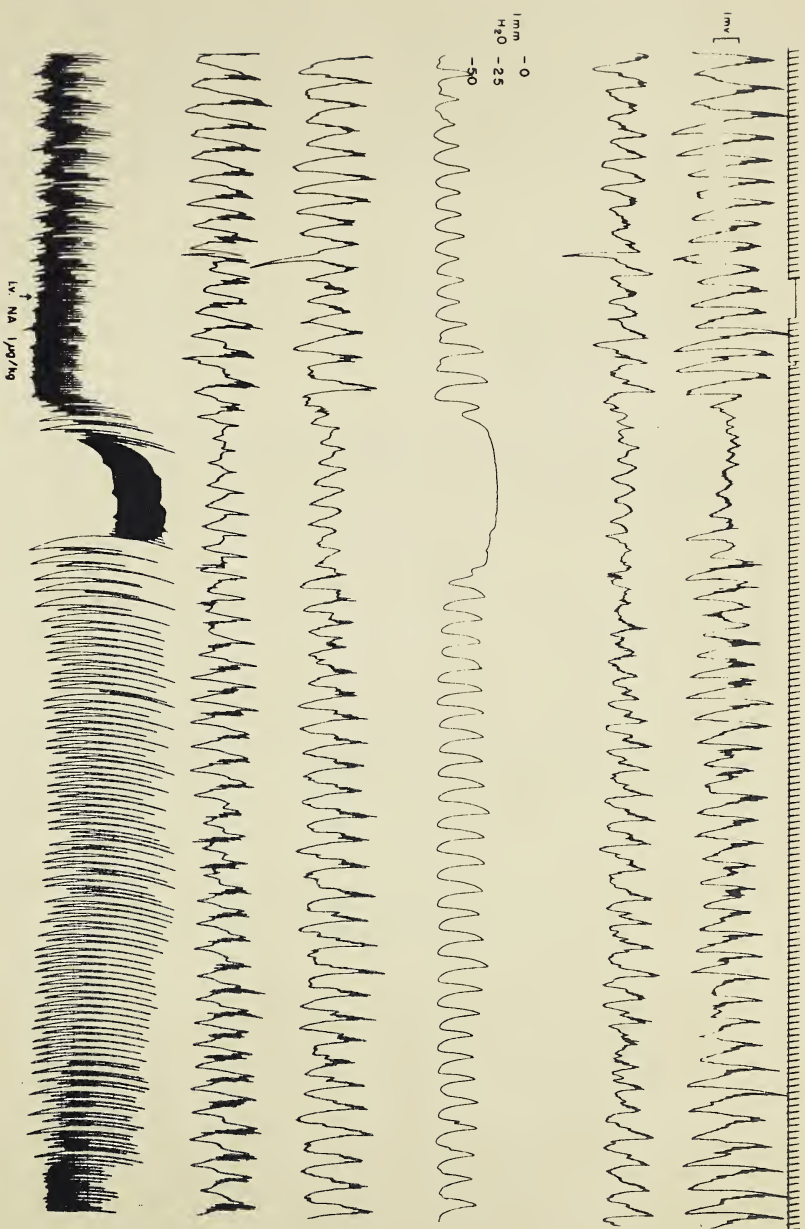


Fig. 1.15 (B). Effects of i.v. NE 1 μ g/kg after Cocaine 2.5 mg/kg. Effects on electrical activity can be seen in all electrodes. Effects on mechanical activity and blood pressure also more pronounced.

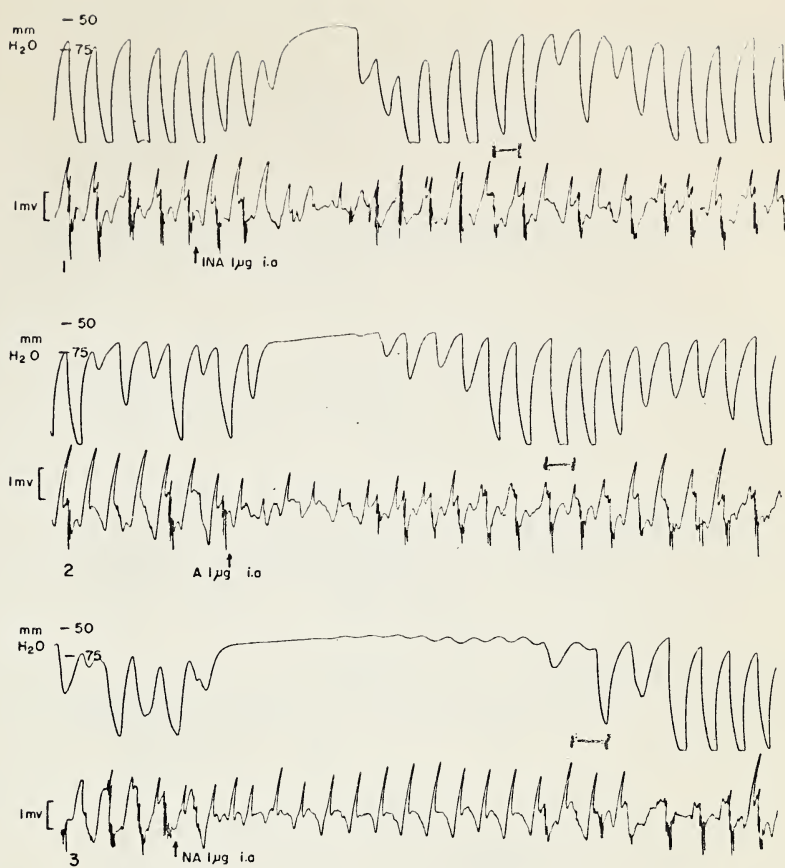


Fig. 1.16 (A). Control perfusions of INA, A, NA. 1 μ g i.o. before Cocaine. Horizontal line = 4 sec.

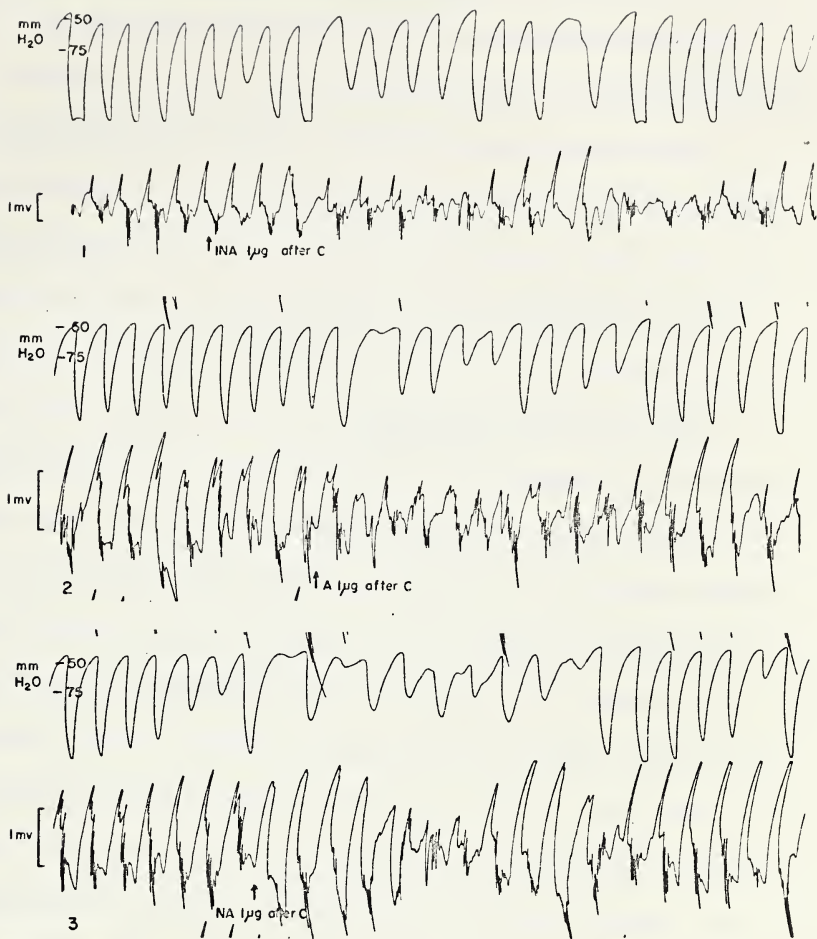


Fig. 1.16 (B). Effect of i.a. perfusion of 1 μ g INA, A, NA after Cocaine 5 mg/kg i.v. Horizontal marking = 4 sec.

DISCUSSION

Effects of Morphine on Intestinal Activity.

It has been shown that morphine produced intestinal spasms in the dog and man (164) and that it had a depressant effect on the guinea pig ileum (165). It has been postulated that morphine interfered with or enhanced the release of acetylcholine from the postganglionic cholinergic nerve endings (164, 165) and that both the stimulant effect as well as the depressant effect were produced by action on the same site i.e., by inhibition or stimulation of release from the postganglionic nerve endings. These effects were not on the ganglia or on preganglionic elements, since they were not blocked by hexamethonium but were blocked by atropine. It has also been postulated (164) that morphine induced stimulation by producing a decrease in the release of inhibitory substances from the postganglionic adrenergic nerve endings in the intestinal wall. Morphine has been shown to have this action on the superior cervical ganglia of the cat (166). The possibility that morphine may act by stimulation of synthesis, release, or by inhibition of release of some other substance besides the transmitters in the intestinal wall (e.g., serotonin, histamine) has not been excluded.

Adrenergic Receptors in the Intestine.

Ahlquist (144) introduced the concept of two kinds of adrenergic receptors to explain the differing potencies of closely related sympathomimetic amines in producing responses in various organs or test systems. These systems showed only two orders of relative potency for the six catecholamines studied and different adrenergic blocking agents were effective against only those

responses produced in test systems showing one of these orders but not the other. Ahlquist classified adrenergic receptors in the intestines as alpha, because of the relative responsiveness of the intestines to epinephrine, norepinephrine and isopropyl-norepinephrine which was of the order shown by alpha-receptors. In fact this was the only inhibitory response of the alpha-receptors. Ahlquist and Levy (58) later solved the problem of why the inhibitory effect of epinephrine in the intestine was not effectively blocked by either the alpha or beta-adrenergic blocking agents given alone. Catecholamines, like phenylephrine, acting primarily on alpha receptors were antagonized by alpha-adrenergic blocking agents. They showed that beta-receptors were also present in the intestines and that their stimulation by isopropyl-norepinephrine also produced inhibition which was blocked by D.C.I. They also showed that the inhibitory effect of epinephrine could only be blocked by a combination of both types of blocking agents.

The experimental results presented here are consistent with the concept of Ahlquist and Levy, that alpha and beta-receptors are both present in the dog small intestine and both are primarily inhibitory. Due to the predominance of the alpha-receptors in the normal dog small intestine the relative responsiveness to i.a. perfusion of E, NE and INE was of the order shown by alpha-receptors. When these were blocked by selective blocking agents the effects on the beta-receptors became more apparent. Complete blockade to the inhibitory effects of E and NE was never obtained with either of the blocking agents used alone. Also after considerable blockade of either of the

receptors, increase in the agonist concentration overcame the block with the appearance of a considerable response. This was not unexpected in the case of the alpha-receptor blockade with tolazoline, considering the reversible competitive nature of the block but it was also seen when irreversible blocking agents like phenoxybenzamine and dibenamine were used. Raising the dose of INE usually also produced a response after beta-receptor blockade by propranolol. However, since all these catecholamines have been postulated to act on both types of receptors (151, 167) (though more selectively on one type) the effect seen on raising the dose of NE after tolazoline may be due to its action on the beta-receptors. Lum et al. (167) have shown that in isolated rabbit jejunum alpha-receptor activity could be impaired by storage in 6-8°C for 24 to 72 hours. In such preparations E and NE produced relaxations which were not blocked by tolazoline but were reduced to the same extent as INE by pronethalol. They assumed that with low doses the primary effect of N and NE were on the alpha-receptors but at high dosages there was considerable effect on both. It has also been postulated that in drug-receptor interactions only a small percentage of the total receptors are occupied in producing even a maximum response (168). Also, Lewis and Miller (169) with tritiated phenoxybenzamine (PBZ-H³) studied receptor occupation and calculated that only a very small portion (0.004%) of the smooth muscle cell of the rabbit seminal vesicle was occupied by PBZ-H³. "Spare receptors" and

"binding sites" could then be responsible for the responses to higher doses of the catecholamines after this blocking agent and possibly even after reversible blocking agents. However, until more conclusive evidence can be presented their existence in the dog small intestine cannot be assumed.

Effect of CA on Intestinal Activity.

Earlier workers (30,31,44) found that epinephrine stopped rhythmic contractions of the intestines but did not affect the slow waves. Later studies have shown that many of the catecholamines produced changes in slow wave amplitude and frequency as well (33,50,53). In the results presented E, NE and INE in doses of 0.5 - 2.0 μg i.a. reduced the amplitude of the slow waves and increased their frequency in most of the preparations studied. This increase in frequency has previously been reported in the duodenum with i.v. infusions (170) as well as with i.a. perfusions in other parts of the small intestines (33). In the antrum the catecholamines produced a decrease in frequency of initial potentials (163,170). In a few cases no change in the amplitude of the slow waves was observed and in such preparations the only effect seen was the disappearance of the fast spikes and the absence of contractions. Smaller doses (0.03 μg - 0.3 μg i.a.) produced little or no effect on the slow waves. In i.v. injections 3 $\mu\text{g}/\text{ml}$ usually produced an effect on the slow waves, spikes and contractions as well as increased the blood pressure. 1 $\mu\text{g}/\text{ml}$ only raised the blood pressure and reduced the contractions. Little or no

effect on the electrical activity was seen in this dose. It is therefore apparent that the effect of the catecholamines on the electrical and mechanical activity of the intestine depended very much on the dose and on the route of administration. Metabolism, and tissue binding would influence the concentration of the drug at the receptor sites and also influence the response of the tissues. The results indicated that a higher concentration of the drug was necessary to inhibit the slow wave activity than that required to abolish fast spikes and contractions.

Are the Effects of Catecholamines on Intestinal Activity Secondary to Ischaemia?

It has been shown that the electrical activity of the intestine is very sensitive to anoxia affecting both the slow wave-complex and reducing its frequency. The possibility therefore remains that the intestinal inhibition shown by i.a. perfusions of NE presented in the results could be secondary to ischaemia caused by the vasoconstrictor effect of the drug. Pituitrin (5 units) considered to produce vasoconstriction by direct action on the vascular smooth muscle, also produced effects similar to NE. However, studies of the time relation as well as other points appearing in the course of the experiments indicated that the effects of catecholamines on intestinal activity was not related to the vasoconstriction. 1. INE which does not produce vasoconstriction also caused intestinal inhibition similar to NE. 2. The time of onset of intestinal inhibition was much earlier (2-4 secs.) than the onset of reduction in blood flow (15-30 secs.). If the inhibition of intestinal

activity was the result of decreased blood flow, there should be a delay in the onset. 3. There is no relationship between reduction in blood flow and the presence or absence of intestinal response. Furthermore, Kewenter (171) has shown that intestinal inhibition produced by low frequency stimulation of the splanchnic nerve was not related to the vasoconstrictor effect. According to Kock (172) "intestinal blood flow usually has to be reduced to $1/3$ or $1/4$ of the resting value before blood flow restriction per se induces a clear-cut intestinal inhibition."

Uptake Storage and Metabolism of Catecholamines.

The fate of catecholamines in the body has been studied extensively and reviews on this subject have been published (173-175). A large part of the circulating E and NE is metabolised either by O-methylation/^{or oxidation.} The remaining portion is rapidly taken up by various tissues and bound in a physiologically inactive form and slowly released (175). This binding has been considered an important mechanism in the inactivation and storage of catecholamines. Studies with tritiated E and NE have shown that NE is more readily taken up by the tissues than is E and the INE is hardly taken up by the tissues at all. Almost all the uptake is by the sympathetic nerve endings and is bound and stored in the granulated vesicles, from which they can be released by stimulation (173). This uptake can be prevented by chronic denervation and by drugs like cocaine and phenoxybenzamine.

Some organs like the heart and spleen can store catecholamines. It has been postulated that the catecholamines are present in at least two metabolic pools (175).

The "active" pool has a rapid turnover and can be released by tyramine, ephedrine and sympathetic nerve stimulation. The "storage" pool is also known as the "tyramine-resistant" pool and is insensitive to the indirectly acting sympathomimetic amines. Process of uptake storage, release and metabolism may complicate studies of dose effect comparisons between the various catecholamines. Drugs that interfere with these processes will also effect the response of these amines by varying the concentration at the receptor sites. Injections of pyrogallol, a COMT inhibitor were found not to influence the intestinal response to i.a. catecholamines showing that metabolism of the perfused drugs at least by COMT was not involved.

The uptake mechanism in the intestine appears to be less efficient than in other organs. Assays of NE conducted in this laboratory have shown a value of $0.1 \mu \text{ mol/gm}$ tissue which is smaller than the value estimated in some other organs (unpublished results). It was also found that i.a. perfusions of tyramine even in large doses did not produce intestinal inhibition (unpublished results) indicating either an absence of release or release of small amounts, too low to produce a detectable effect.

Potentiation by Cocaine.

Cocaine has been shown to potentiate some of the effects of E and NE on sympathetically innervated organs (59,60). Several hypotheses have been presented as the mechanism for this action. Some workers suggest that cocaine may act by preventing the destruction of injected catecholamines as well as catecholamines released from the sympathetic nerve endings (176). Others suggest that prevention of

uptake and release might be involved (177,178). This results in higher concentrations of catecholamines in the vicinity of the receptor sites. Whitby et al. (59) conducted studies with tritiated NE (H^3NE) in the heart spleen and adrenals (three tissues which have the greatest uptake) and showed that cocaine produced a marked reduction in the H^3NE content. He also showed that cocaine markedly reduced uptake of NE and suggested that cocaine interfered with binding. Muscholl showed correlation between uptake and potentiation in the heart and spleen and the specificity of cocaine in producing these actions (60). He showed that cocaine 20 mgm/Kg reduced the uptake in these organs by 50%.

In the results presented cocaine was found not to potentiate i.a. perfusions of E and NE, though i.v. injections of NE were potentiated. While this evidence that cocaine-sensitive uptake of catecholamines does not influence their actions in the intestines, is consistent with morphological evidence of lack of close innervation of muscle cells by nerves, it does not prove that intestinal sympathetic nerves are insensitive to cocaine. Thoenen (179) had emphasized the importance of distance between receptors and stores. If the NE stores were at some distance from the receptors, blockade of uptake at the stores will influence the concentration at the receptor sites less than if the stores were in close proximity to the receptor sites. Norberg (16) has shown that there are few adrenergic terminals in the vicinity of the muscle cells, the actual nerve endings being situated in the myenteric plexus. However, recently the alpha-receptors have been postulated

to be in the postganglionic nerve endings in the myenteric plexus in the cat, guinea pig and the rat (160-162). If that were the case in the dog, cocaine might be expected to inhibit uptake of NE into these endings and potentiate any effect of catecholamines on nearby receptors. However, the actual location of the alpha-receptors in the dog intestine has not been established. At the present time the absence of alpha-receptor potentiation by cocaine can be explained as the possible consequence of small uptake capacity of the intestinal sympathetic nerve endings or of a large distance between these endings and the receptor, or of the sluggish uptake and release or the combination of all three. The potentiation of the i.v. effects of NE by cocaine is probably due to reduced uptake in the other tissues resulting in a larger concentration of the drug reaching the intestines.

CONCLUSIONS.

1. Adrenergic receptors in the intestines are predominantly alpha, but both alpha and beta-receptors are present and stimulation of either receptor produced inhibition.
2. E, NE and INE produced intestinal inhibition by stimulation of these receptors. Each drug acted selectively on one type of receptor but had a variable effect on the other.
3. Effects of the catecholamines on intestinal activity were not the result of ischaemia due to vasoconstriction though the effects may be additive in large doses.
4. Cocaine did not potentiate i.a. effects of NE but did potentiate the i.v. effects.

SECTION II

EFFECT OF ISCHAEMIA ON THE ELECTRICAL AND MECHANICAL ACTIVITY OF THE DOG SMALL INTESTINE

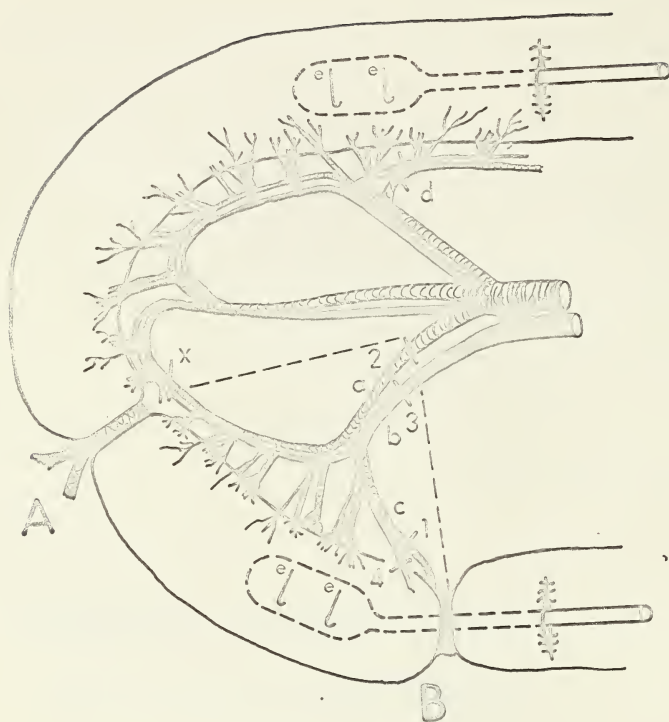


Fig. 2.1. Diagrammatic representation of the jejunal loop, showing the experimental segment A B, the control segment proximal to it, and the arterial arcade. 'A' and 'B' are the areas where rubber ligatures were tied. The dotted line in each segment and the hooked lines 'e' denotes balloons and electrodes that will be placed at the time of recording. The various letters and numbers indicate the vessels and site of ligatures or clamps applied, (See text). The experimental segment was isolated by cutting along the dotted line on either side of the vessels.

OBJECT OF STUDY

To test the hypothesis that ischaemia of 4 hours duration effectively and selectively destroys the ganglion cells of the myenteric plexus.

I. ACUTE IN VIVO EXPERIMENTS (6)

In this series, the experiments were carried out to study the changes in resting and drug induced electrical and mechanical activity after 4 hours ischaemia, and to relate these changes to nerve and/or muscle damage.

METHODS

Surgery. Male dogs weighing 10-15 kg. were used. Female dogs were not used because the abundance of mesenteric fat in many female dogs makes ligation of the small vessels difficult.

The dog was prepared and anaesthetised with the urethane-chlorolose mixture and the abdomen opened as in Section I. Two adjacent segments of jejunum with an arterial arcade in between were chosen. This ensured an adequate blood supply to the area at the end of surgery. The proximal segment served as a control and the distal segment was later made ischaemic. The distribution of the arterial and venous arcade in the loop of jejunum, and the vessels used for clamping and cannulation are shown in Fig. 2.1. The main artery 'a' and vein 'b' distributing blood to the distal experimental segment 'AB' was carefully dissected and cleaned of the surrounding tissue and nerves. All the mesenteric nerves that could be seen were cut. The mesenteric tissue on either side of the vessels was tied with cotton thread to make sure that there was no leakage

of blood to the segment from the very small vessels running in the mesentery. A small artery 'c' from the vasa recta supplying the part of the intestine, adjacent to one end of the segment was also isolated and ligatured at 'l'. A cannula was inserted in the artery proximal to the ligature and directed towards the main artery and opposite to the direction of blood flow. The same cannula could be used for perfusion of drugs and for flushing out the blood from the segment to produce ischaemia. During the period of i.a. perfusions to the segment with intact blood supply the main artery was clamped so that an adequate concentration of the drug reached the segment. A volume of 2 mls. of saline solution flushed into the cannula produced a blanched area of 2-3 cms and delineated the area that was perfused. Two silver wire electrodes 'ee' of the type used in Section I were placed in the perfused area. A condom balloon was introduced into the lumen through a distal stab incision and the balloon held in place by sutures at the wound. The proximal segment was cannulated in the same way at 'd' and the electrodes 'ee' inserted. In this segment the balloon was introduced through a stab incision made proximal to the area under study.

Control recording of the electrical and mechanical activity was made and the effects of i.a. perfusions of drugs selected for stimulation of nerve or muscle were studied. The perfusions were made at 10 minute intervals between series and 5 minute intervals between doses. The distal segment was then made ischaemic according to the technique described by Hukuhara et al. (34). The small vessels of the arcade on the side opposite to the cannulation were ligated

at 'x'. Rubber tubing (size 1/8" ID x 1/32" wall) was used as ligatures and the two ends of the segment 'A' and 'B' were tied. The mesentery on either side was cut as far up as the place where it had previously been tied on either side of the main artery. In this way the loop was completely isolated except for the artery and vein connected to it. The main artery was clamped at '2' with a 1¹/₂" bulldog clamp. Non-oxygenated, glucose-free Krebs Ringer solution was injected through the cannula at moderate speed. The solution perfused the blood vessels of the loop via the arteries and flowed out through the vein, finally resulting in complete expulsion of all the blood in the loop. The segment then became entirely white and the blood vessels were filled with clear solution. This was usually accomplished with 30-60 mls of solution but in a few cases up to 100 mls of solution were required. The vein draining the segment was clamped at '3' as was its branch accompanying the artery 'c' at '4'. A picture of one such preparation from a later series is shown in Fig.2.10. The jejunal loop was carefully replaced in the abdominal cavity and the segment kept in a state of complete ischaemia for 4 hours.

At the end of 4 hours the segment was still white and appeared flaccid. All preparations that were pink with blood or cyanotic were rejected. The ligatures were untied, the clamps on the arteries and veins removed and the blood supply to the segment was restored. The segment rapidly filled with blood and the colour of the intestine returned to normal within a few minutes. 30 minutes after the re-establishment of blood supply i.a. perfusions were repeated

and the electrical and mechanical activity in the two segments recorded. Comparison of the electrical and mechanical activity was made in the experimental segment before and after 4 hours ischaemia. The same comparison was made in the control segment where no actual ischaemia had been produced. This was done to eliminate changes due to anaesthesia, time, and handling. The differences in responses in the experimental segment relative to the differences in responses in the control segment were considered as those due to ischaemia.

Recording Systems. An Offner dynograph of the same type used in Section I was used for recording the electrical and mechanical activity in these experiments.

Drug Solutions Used.

Krebs Ringer solution of the same composition described in Section I was used for dilution of stock solutions and also for flushing the drug from the cannula line. This solution was aerated with 95% oxygen and 5% carbon dioxide mixture. Non-oxygenated Krebs Ringer solution used for flushing out the blood from the segment did not contain glucose and was not aerated. The temperature of these solutions was kept at 37°C.

Stock solutions were made, containing 1 mgm/ml of drug calculated either as a salt or as its base. These were diluted, so that 1 ml of the diluted solution contained the required concentration of the drug. This was the volume of the solution always used for perfusion and was flushed in with 1 ml of Krebs Ringer solution. Drugs were:

1. Phenylbiguanide (P.D.G.) 10 µg/ml; 50 µg/ml.
2. Methacholine (Mch) 0.025 µg/ml; 0.1 µg/ml;
0.25 µg/ml (calculated as the base).

3. Serotonin Creatinine Sulphate (5-HT) $0.1 \mu\text{g/ml}$;
 $0.5 \mu\text{g/ml}$ (calculated as the base).
4. Dimethylphenylpiperazinium (D.M.P.P.) $2.5 \mu\text{g/ml}$;
 $5 \mu\text{g/ml}$.
5. Nicotine (Nict) $5 \mu\text{g/ml}$.
6. Double NaCl, Krebs Ringer solution.

This was made by doubling the concentration of NaCl in the normal Krebs Ringer solution without changing other constituents.

7. Pure KCl-Ringer solution. Krebs Ringer solution was modified substituting 115.5 meq KCl for 115.5 meq NaCl.

Assumptions Made in the Use of the Drugs

1. P.D.G. stimulates the mechanoreceptors and activates the intrinsic reflex (8, 102).
2. D.M.P.P. stimulates ganglion cells selectively (135).
3. Mch stimulates muscle cells directly.
4. 5-HT exerts its action indirectly on the nerve-endings as well as directly on the muscle.
5. Double NaCl Krebs Ringer solution stimulates the nerve endings to release Ach (102).
6. KCl-Ringer stimulates the muscle directly at least in the high concentration used (102).
7. Nicotine is a ganglionic stimulant. There were serious problems in its use owing to the ease with which tachyphylaxis occurred and this antagonised drugs like D.M.P.P.

If the ganglion cells have been effectively destroyed:

1. Responses due to P.D.G. and D.M.P.P. in the experimental segments should be absent or less when compared to the responses before ischaemia.
2. No such differences should be seen in the control segment or the differences should be much less than seen in the experimental segment.

If the nerve-endings are affected:

3. Responses to 5-HT should be reduced in the experimental segment.
4. Responses to the Double NaCl-Krebs Ringer should be present in the control and absent in the experimental segment.
5. Responses to Mch should show little or no difference in both segments.
6. Responses to KCl-Ringer should be present equally in both segments.

Assessment of Results.

The changes in the responses in vivo of the control segment and of the experimental segment after ischaemia in the latter were computed. Then the change in response of the experimental segment was subtracted from the change in response of the control segment and the mean and standard error of the resultant values computed. When these means were significantly ($P \leq 0.05$) greater than zero the responses in experimental segments were considered to be reliably more depressed than in control segments. This is a crude statistical analysis in which the outcome is weighted against achievement of statistically significant differences which nonetheless, were found. The responses in vitro of experimental and control segments were analysed by determining

the difference in the responses of each pair and computing whether the resultant values were significantly different from zero ($P \leq 0.05$).

RESULTS (6 experiments)

I. Electrical and Mechanical Activity.

Control Recording. The electrical and mechanical activity of the two segments before ischaemia, showed normal activity similar to the type described in Section I.

After 4 hours. (Here as well as in the subsequent experiments, this expression means that during this period the experimental segment was kept ischaemic and no intentional ischaemia was produced in the control segment). Observations were made 30 minutes after re-establishment of blood supply. The control segment in which no actual ischaemia was produced appeared normal in colour and tone. Brisk reflex responses were seen on pressure or on stroking the muscle. The electrical and mechanical activity recorded, was very little different from that recorded 4 hours before. The appearance of the experimental segments in these acute experiments varied (this series as well as the next). In the majority of the dogs, in which

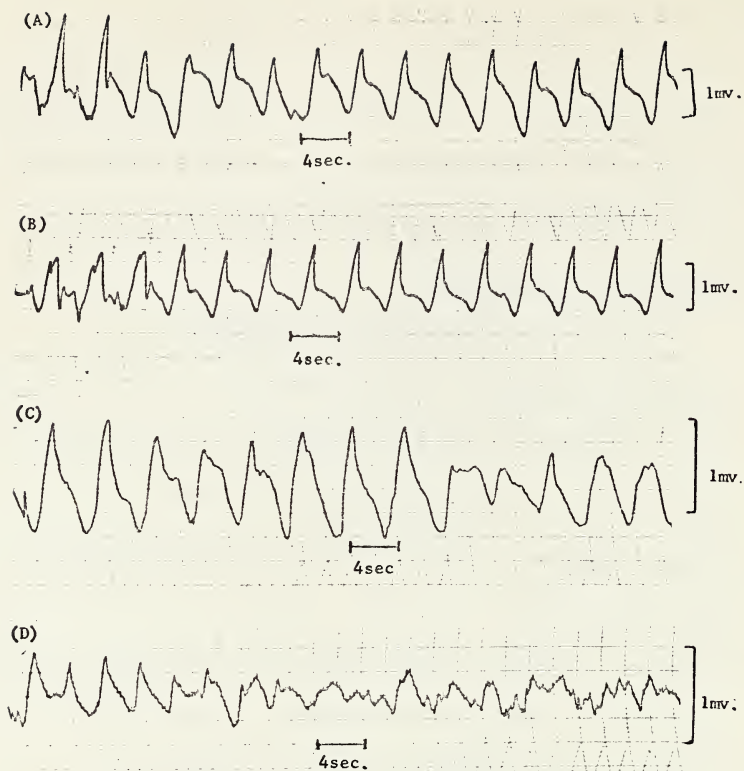


Fig. 2.2. Electrical activity of the two jejunal segments before and after 4 hours.

(A) Control (above) and (B) experimental (below) segments before 4 hours.

(C) Control (above) and (D) experimental (below) segments after 4 hours.

not more than 50 mls of solution had been used to flush out the blood from the segment and in which surgery had been accomplished with minimum handling, the segment appeared normal in colour and was only slightly flaccid. Sluggish responses were seen on extreme pressure or hard stroking. In few others where the surgery required more handling or when a larger volume of solution had been used to completely flush the blood out of the segment, a complete loss of tone was seen. Such segments had poor venous drainage and appeared slightly congested. Large amounts of exudates were present in most of these preparations.

The mechanical activity recorded in the experimental segment after ischaemia was at a minimum and the electrical activity was poor in all the six dogs of the series. Slow waves appeared periodically at varying intervals and were of low amplitude (<0.5 mv) and usually distorted Fig. 2.2. Because of their irregular appearance it was not possible to study either their frequency or propagation in this series. Spontaneous spikes were never seen nor could they be induced by morphine or eserine in this series.

Because of the poor and inconsistent electrical activity after several hours in these acutely prepared dogs, evaluation of results in this series was based on changes in the mechanical activity produced by i.a. perfusion of drugs before and after ischaemia.

TABLE 2.1

RESPONSES OF THE CONTROL AND EXPERIMENTAL

JEJUNAL SEGMENTS TO PHENYLDIGUANIDE*

C = Control, E = Experimental, NR = No Response

↓ = Preceded by Relaxation

Experiment No.		Maximum height of contraction (mm of H ₂ O)	
		Before 4 hrs.	After 4 hrs.
1	C	100.0	62.5
	E	87.5	NR
2	C	↓ 37.5	100.0
	E	↓ 25.0	NR
3	C	37.5	50.0
	E	62.5	NR
4	C	↓ 37.5	50.0
	E	↓ 75.0	NR
5	C	**112.5	↓ 25.0
	E	37.5	NR
6	C	↓ 112.5	↓ 87.5
	E	↓ 87.5	NR

* P.D.G. 10 µg/ml was perfused i.a. and flushed in with 1 ml Krebs Ringer solution.

** Response in the control segment was reduced 75%. Because of the atypical response of Dog No. 5, the responses were not significantly more depressed in ischaemic than in control segments. If however, all changes were normalized by taking initial responses as 100% and computing percentage change, the differences were significant.

II. Responses to Drugs.

It was found that contractile responses of the experimental segment were not related to the appearance of good slow waves. Responses of reasonable dimensions could be seen in mechanical records in preparations with very poor electrical activity. Also the magnitude of the responses in the two segments often differed. For this reason, each segment was used as its own control. Difference in responses of one segment was compared with difference in responses of the other segment. As mentioned earlier, the difference in responses of the experimental segment relative to those in the control segment were considered to be the result of ischaemia.

(1) Phenyldiguanide.

Drug solution used was P.D.G. 10 $\mu\text{g.}/\text{ml}$ and 50 $\mu\text{g.}/\text{ml}$. 1 ml of drug solution was used and flushed in with 1 ml Krebs Ringer solution (see methods).

Control Reading. (before 4 hours ischaemia) P.D.G. caused contractions of 25-100 mm H_2O in both segments. A few spikes were evoked in the electrical records. In a number of experiments (3) the contractions were preceded by small relaxations of about 25 mm. H_2O (duration 2-4 secs) in both the control and the experimental segments (Fig. 2.3).

After 4 Hours. The responses to P.D.G. in the control segments of two dogs were reduced to 25% and 75%. The responses in the other four preparations were approximately equal to, or greater than those observed 4 hours previously. No responses were seen after ischaemia in any of the experimental segments. Results of the experiments with P.D.G. on six dogs are given in Table 2.1.

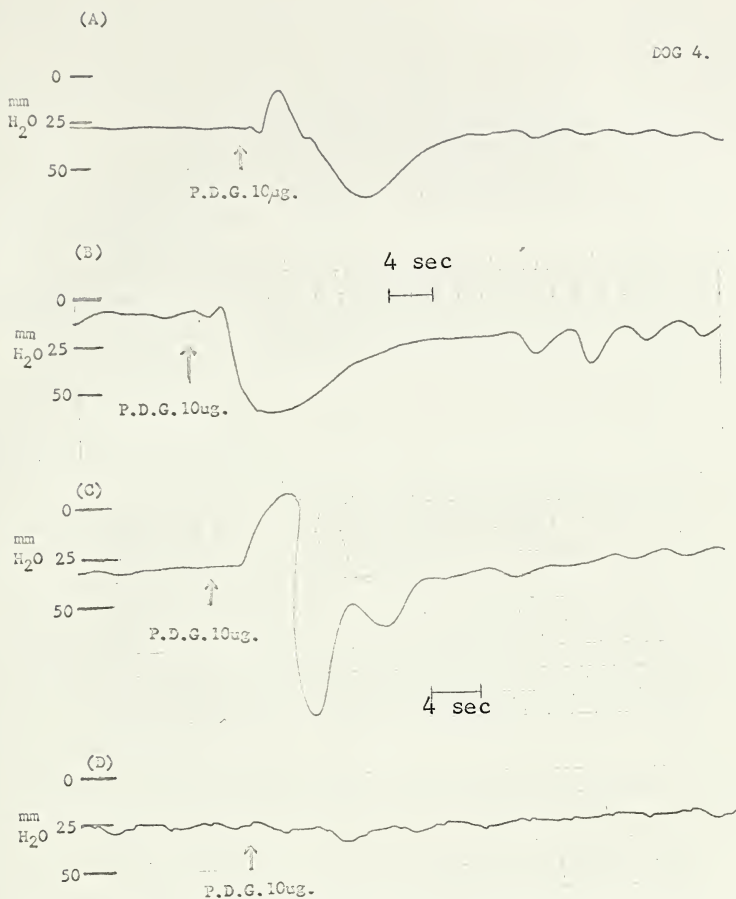


Fig. 2.3. Mechanical responses of the two jejunal segments to i.a perfusion of P.D.G. 10 μ g before and after 4 hours.

- (A) Control segment before 4 hours (B) after 4 hours. Approximately equal responses.
- (C) Response of the experimental segment before 4 hours and (D) after 4 hours showing an absence of response.

RESPONSES OF CONTROL AND EXPERIMENTAL SEGMENTS

OF JEJUNUM TO METHACHOLINE*

C = Control, E = Experimental, After = After 4 hrs, Before = Before 4 hrs.

ND = Not Done, NR = No Responses ↓ = Preceded by Relaxation

Dog No.	Maximum height of contraction to different doses of Mch.					
	0.025 µg		0.1 µg		0.25 µg	
	Before	After	Before	After	Before	After
	H ₂ O mm	mm	mm	mm	mm	mm
1	C	50.0	25.0	ND	62.5	ND
	E	50.0	NR	ND	37.5	ND
2	C	75.0	62.5	112.5	100.0	112.5
	E	75.0	NR	87.5	50.0	75.0
3	C	50.0	50.0	100.0	87.5	100.0
	E	75.0	25.0	100.0	37.5	62.5
4	C	↓ 25.0	37.5	↓ 75.0	100.0	100.0
	E	↓ 25.0	NR	50.0	25.0	50.0
5	C	↓ 75.0	37.5	62.5	100.0	112.5
	E	37.5	NR	50.0	37.5	50.0
6	C	50.0	25.0	75.0	62.5	100.0
	E	25.0	NR	62.5	25.0	25.0

* The required concentration of the drug given above was perfused i.a. in 1 ml volume and flushed with 1 ml Krebs Ringer solution. The decrease in response to Mch following ischaemia of the experimental segment was significantly (at $P \leq 0.05$) greater than that in the control segment.

The changes in the mechanical activity of one such preparation are shown in Fig. 2.3. In two experiments the dose of P.D.G. was raised to 50 μg with no response in the experimental segment. The response in the control segment was approximately the same as that produced by 10 μg .

(2) Methacholine.

Drug solutions used was Mch 0.025 $\mu\text{g}/\text{ml}$, 0.1 $\mu\text{g}/\text{ml}$ and 0.25 $\mu\text{g}/\text{ml}$ calculated as the base. 1 ml of the solution was used for perfusion and was flushed in with 1 ml of Krebs Ringer solution.

Control Recording. Mch 0.025 μg produced a contraction of about 25-50 mm H_2O in five out of six experiments. This contraction was accompanied by a few spikes in the electrical record. Larger doses produced bigger contractions of 75->100 mm H_2O and were accompanied by an increase in the number of spikes.

After 4 Hours. The responses in the control segment, before and after 4 hours were approximately equal. In the experimental segment little or no response was seen with Mch. 0.025 μg . Raising the dose of Mch to 0.1 μg and 0.25 μg produced responses that were approximately equal to those produced by the smaller dose before ischaemia. Results of these experiments with Mch. in doses of 0.025 μg , 0.1 μg and 0.25 μg are given in Table 2.2. Fig. 2.4 shows approximately equal responses produced by equal doses (0.025 μg) of Mch before and after 4 hours in the control segment. In the experimental segment after ischaemia, a dose of 0.25 μg was required to produce the same magnitude of contraction as 0.025 μg before ischaemia.



Fig. 2.4. Mechanical responses of the two jejunal segments to Mch before and after 4 hours.

A, B, Equal responses of the control segments to i.a. perfusion of equal doses of Mch (0.025 μ g) before and after 4 hours.

(C) Response of the experimental segment to i.a. Mch. 0.025 μ g before ischaemia; (D) after ischaemia the dose of Mch 0.25 μ g produced approximately the same response. Horizontal Line = 4 sec.

TABLE 2.3

RESPONSE OF THE CONTROL AND EXPERIMENTAL JEJUNAL SEGMENTS

TO 5-HT BEFORE AND AFTER 4 HOURS

C = Control, E = Experimental, Before = Before 4 hrs.,

After = After 4 hrs., ND = Not Done, NR = No Response

Dog No.		Height of contraction in mm H ₂ O			
		0.1 µg 5-HT		0.5 µg 5-HT	
		Before	After	Before	After
1	C	75.0	NR	25.0	62.5
	E	75.0	NR	62.5	NR
2	C	25.0	25.0	87.5	37.5
	E	25.0	NR	100.0	25.0
3	C	75.0	25.0	75.0	37.5
	E	50.0	NR	62.5	25.0
4	C	100.0	NR	100.0	25.0
	E	62.5	NR	75.0	NR
5	C	25.0	25.0	62.5	62.5
	E	62.5	25.0	NR	NR
6	C	87.5	50.0	ND	ND
	E	50.0	25.0	ND	ND

(3) 5-Hydroxytryptamine.

Drug solutions used was 5-HT 0.1 μ g and 0.5 μ g calculated as the base. 1 ml of solution was perfused and flushed in with 1 ml of Krebs Ringer solution.

Responses to 5-HT were very variable and can be seen from the results presented in Table 2.3.

Control Recording. In the control recording both 0.1 μ g and 0.5 μ g of 5-HT produced good contractions and a few spikes in both the control and in the experimental segments.

After 4 Hours. Little or no response was seen in either segment and responses to this drug did not serve to differentiate between the ischaemic and control segments.

(4) Dimethylphenylpiperazinium and nicotine.

Drug solutions D.M.P.P. 2.5 μ g/ml and 5 μ g/ml. Nict. 5 μ g/ml. 1 ml of solution was perfused and flushed in with 1 ml Krebs Ringer solution.

Control Recording. Intra-arterial perfusions of 2.5 μ g D.M.P.P. produced contractions ranging from 25-100 mm H₂O with a variable number of spikes in the electrical record. Raising the dose to 5 μ g did not greatly increase the magnitude of the contraction but the duration was longer and there was an increase in the number of spikes in the electrical record. In four out of six experiments the contractions were preceded by relaxations of 25 mm H₂O lasting 4-8 secs. This was observed in both the control and the experimental segments. When the relaxation was more pronounced the contractions observed were very small.

After 4 Hours. After 4 hours, contractile responses to 2.5 μ g D.M.P.P. were not obtained in the experimental segment in any of the six dogs. A small response occurred in two out of six

TABLE 2.4

RESPONSES OF THE CONTROL AND EXPERIMENTAL SEGMENTS
OF JEJUNUM TO D.M.P.P. AND NICT.*

C = Control, E = Experimental, After = After 4 hrs, Before = Before 4 hrs,
NR = No Response, ND = Not Done, Preceded by Relaxation.

Dog No.	Maximum + height of contraction in mm H ₂ O				NICT.	
	D.M.P.P.		D.M.P.P.			
	2.5 µg		5.0 µg		5.0 µg	
	Before	After	Before	After	Before	After
1	C 87.5	50.0	ND	ND	ND	ND
	E 62.5	NR	ND	ND	ND	ND
2	C 37.5	62.5	25.0	50.0	50.0	100.0
	E 37.5	NR	50.0	25.0	75.0	25.0
3	C 25.0	NR	25.0	25.0	112.5	37.5
	E 87.5	NR	25.0	37.5	112.5	25.0
4	C 75.0	25.0	75.0	25.0	112.5	87.5
	E 50.0	NR	62.5	NR	37.5	NR
5	C 100.0	37.5	87.5	NR	62.5	37.5
	E 62.5	NR	50.0	NR	50.0	NR
6	C 25.0	25.0	37.5	25.0	87.5	100.0
	E 62.5	NR	87.5	NR	75.0	NR

* The drugs were given i.a. in 1 ml of perfusion volume.
The difference in reduction of response between the control and experimental segments was statistically significant at P = 0.05 with D.M.P.P. 2.5 µg (the response of the experimental segment being more reduced). The results were not significantly different with D.M.P.P. 5 µg or Nict. 5 µg.

experiments when the dose was raised to 5 μ g. The evaluation of the results with D.M.P.P. was made difficult by the absence of a clear response in some of the control segments. Fig. 2.5 shows the response in the control segment after 4 hours, reduced to 25% of the contraction obtained before the 4 hours. There was no response in the experimental segment. With repeated perfusions, tachyphylaxis was common.

Because of these variable results with D.M.P.P., nicotine 5 μ g was given in the latter five dogs, as an added test for the presence of functional nerves. D.M.P.P. was given before nicotine and an interval of 30 minutes was allowed between the two drugs. In the control records, nicotine produced contractions of 50-100 mm H₂O in both segments. Relaxation preceded the contraction in three experiments. Contractions were usually accompanied by spikes in the electrical record before the 4 hour period of ischaemia.

After 4 Hours. In four out of five dogs, responses in the control segment were approximately the same, before and after the 4 hour period. In one dog the magnitude of contraction was reduced to 1/3 of the control record. In the experimental segment no response was seen in three dogs and a slight response seen in two. Results of these experiments are given in Table 2.4 Fig. 2.6 shows the response of the two segments before and after ischaemia.

(5) Double NaCl Krebs Ringer Solution.

Krebs Ringer solutions modified by doubling the concentration of NaCl but not of other constituents was perfused i.a. in both the control and the experimental segments after 4 hours ischaemia. This has been shown to stimulate the nerve endings causing a release of Ach, and an increase in intestinal activity (102). In three...(Continues on next page)



Fig. 2.5. Mechanical responses of the two jejunal segments to DMPP 2.5 μ g before and after 4 hours. A, B, Responses of control segment before and after 4 hours. Response in B is much less (about 25%) than the response in A. (C) Response of experimental segment before and after (D) after ischaemia. No response in D. The contractions in A and C were preceded by relaxations.
Horizontal Line = 4 sec.

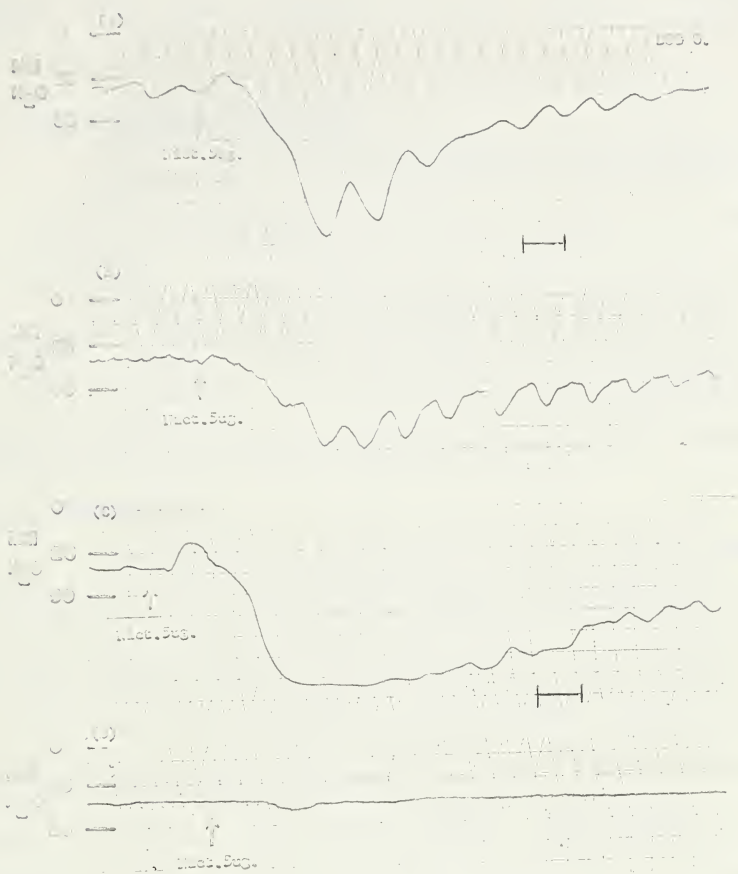


Fig. 2.6. Mechanical responses of the two jejunal segments to Nicotine 5 μ g.
 A = Response in the control segment before 4 hours.
 (B) After 4 hours.
 (C) Response in the experimental segment before 4 hours and (D) after 4 hours. No response occurred in D.
 Horizontal Line = 4 sec.

experiments 5-10 mls of Double NaCl-Ringer solution produced a contraction in the control segment and not in the experimental segment. There was no response in either segment in the other three experiments.

(6) Pure KCl-Ringer Solution.

Krebs Ringer solution modified by substituting KCl 115.5 meq for 115.5 meq NaCl was used to estimate the extent of muscle damage. It was administered only at the end of the experiments. 5 mls of Pure KCl Ringer solution were injected i.a. into the segments. The responses of the experimental segments were less than the responses of the control segment.

III. Conclusions.

The results of experiments of this series appeared to indicate that there was possible damage or destruction of the neuronal elements of the intrinsic plexus. The responses to all drugs which were assumed to act on ganglia (D.M.P.P. and nicotine) were either abolished or reduced in the experimental segment relative to the control segment. The response to P.D.G. assumed to act on mechanoreceptors and therefore to require the presence of functioning nerves was also absent in the experimental segments. The extent of nerve damage and whether it was permanent or reversible could not be estimated. However, the reduction in the responses to Mch and KCl in the experimental segments also indicated concomitant damage of the muscle cells. The possibility was further supported by the absence of visible spontaneous as well as by the poor recorded electrical activity in the form of small distorted and irregular slow

waves and the complete absence of spontaneous and induced spikes. These activities are considered myogenic in origin. In vitro studies on these tissues showed no response to any drugs. At the end of the experiments about 3 cm of each segment was removed and fixed in 10% basic formalin and sent to the Pathology Laboratory for histological studies (see later).

II. ACUTE IN VITRO EXPERIMENTS (6)

These experiments were designed to find out the extent of damage in the muscles due to ischaemia. To avoid possible oedema, and changes due to drugs and prolonged exposure, i.e. perfusions were not done in these preparations.

METHODS

S u r g e r y. The technique for producing ischaemia was similar to the procedure described for acute in vivo experiments. At the end of 4 hours during which the experimental segment was kept in a state of complete ischaemia, the segments were allowed to recover for 30 minutes after restoration of the blood supply. The control and the experimental segments were removed and immediately placed in oxygenated Krebs Ringer solution.

P r e p a r a t i o n o f T i s s u e s a n d O r g a n B a t h. Intestinal strips measuring 0.3-0.5 cm x 3 cms were prepared from the control and experimental segments. The mucous membrane was gently scraped away and the strips were suspended in a 10 ml organ bath filled with oxygenated Krebs Ringer solution. The temperature was maintained at 37°C throughout the experiment. The lower end of the intestinal strip was attached to the hook of a small glass tube through which 95% O₂ and 5% CO₂ mixture was bubbled. The upper end was connected by a cotton

thread to a frontal writing lever under 1 gram tension. Isotonic contractions were recorded with 3 fold magnification on a smoked drum.

D r u g Solutions Used.

1. Krebs Ringer solutions (as in the previous series)
2. Mch - Stock solution prepared contained 1mgm/ml of methacholine base. Dilutions were made to obtain concentrations of 1.0 μ g/ml and 0.1 μ g/ml.
3. D.M.P.P. - Stock solution prepared contained 1 mgm/ml Dimethylphenylpiperazinium. 0.2-0.4 mls were added to the bath.
4. Nicotine - Stock solution of 1% (10 mg/ml) was prepared. Dilutions were made to obtain a concentration of 100 μ g/ml.
5. Double NaCl-Ringer solution - This was prepared as previously described.
6. Pure KCl-Ringer solution - Also described previously.

R e c o r d i n g of Drug Responses. The responses of the control and experimental strips to five concentrations of Mch were studied. The concentrations used were 0.001 μ g/ml; 0.0025 μ g/ml; 0.005 μ g/ml; 0.0075 μ g/ml and 0.01 μ g/ml. The minimum concentration required to produce a response was particularly noted. In two preparations where there was no response to the above concentrations, the dose was raised to a higher range (0.01 μ g to 0.1 μ g). The height of contraction in millimeters was then plotted against log concentration.

Responses of the strips to D.M.P.P. 20 μ g/ml; and nicotine 5 μ g/ml were then studied. The contractions produced

by changing the bathing medium to Double NaCl Krebs Ringer solution and Pure KCl-Ringer solution were also recorded.

RESULTS

Spontaneous Activity. Pendulum movements were observed in all the control strips, 15-30 minutes after the tissue had been suspended. Pendulum movements of the type seen in the control strips were recorded in only one of the six experimental strips. In two other strips spontaneous contractions did occur in the form of a slow rise and fall of the base line.

Responses to Methacholine. In all six preparations, contractile responses to Mch in the experimental strips appeared to be less than the responses in the control strips in the same dose. Fig. 2.7; (a-f). The threshold dose in the experimental strip was doubled in one preparation Fig. 2.7 (c) and increased five times in another Fig. 2.7 (d). In both these preparations there was no response to Mch in the range 0.001 $\mu\text{g/ml}$ to .01 $\mu\text{g/ml}$ as in the other preparations. Responses were obtained only when the dose of Mch was raised to the range of .01 $\mu\text{g/ml}$ to 0.1 $\mu\text{g/ml}$. In the other four preparations, the threshold dose was approximately the same but the magnitude of the contractions in the experimental strip was clearly less. The maximum response was also reduced in all six preparations. The heights of the contractions in responses to different doses are tabulated in Table 2.5.

Responses to Drugs Which Stimulate Nerve Elements. D.M.P.P. 40 $\mu\text{g/ml}$ and nicotine 5 $\mu\text{g/ml}$ were used as ganglion stimulants, and Double NaCl Krebs Ringer solution was used for stimulating the nerve endings. Pure KCl-Ringer was used to show the contractile integrity of the muscle. The experimental strips of

Fig. 2.7 (a.f.) In vitro responses of control and experimental jejunal strips of 6 dogs to Mch.

The height of contraction in mm is plotted against log dose concentration.

The responses of the experimental segment (●) are lower than those of the control (○) in the same dose range.

Dog 3 and 4 (c) (d) have no response to .001-.01 μ g/ml. and the dose range had to be increased to .01 - .1 μ g/ml.

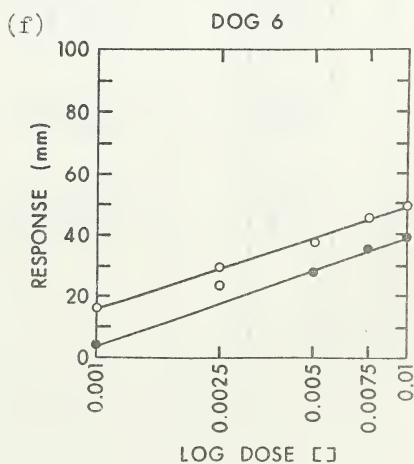
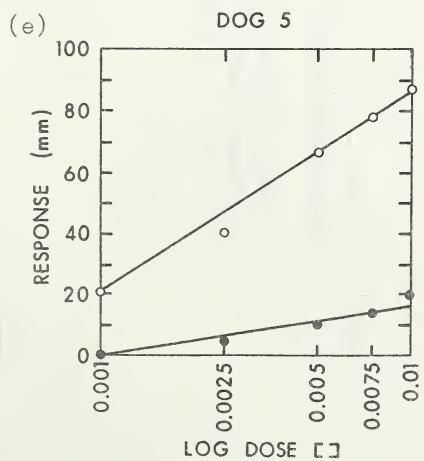
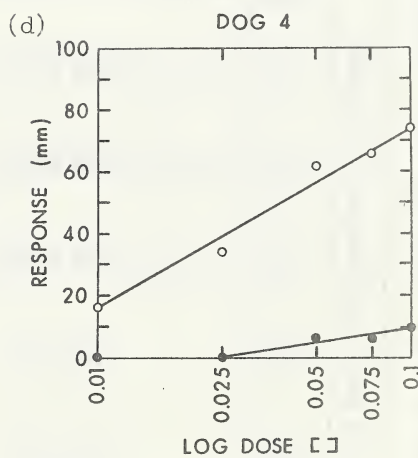
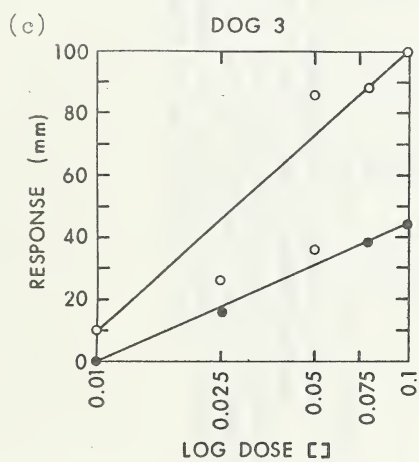
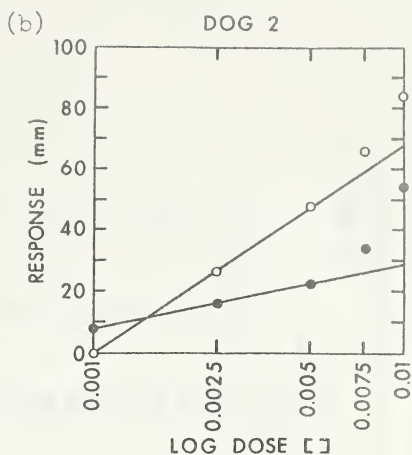
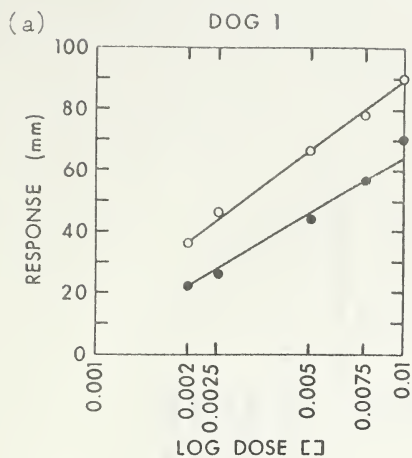


TABLE 2.5

IN VITRO RESPONSES OF THE JEJUNAL STRIPS TO METHACHOLINE*

C = Control, E = Experimental, ND = Not Done, NR = No Response

Dog No.	Height of contraction in mm H ₂ O										
	** .001 μg/ml	.0025 μg/ml	.005 μg/ml	.0075 μg/ml	.01 μg/ml	.01 μg/ml	.025 μg/ml	.05 μg/ml	.075 μg/ml	.1 μg/ml	
1	C NR	3.8	6.6	7.8	9.0						
	E NR	2.4	4.4	5.6	7.6						
2	C NR	2.6	4.8	6.6	8.4						
	E 0.8	ND	2.2	3.4	5.4						
3	C NR	NR	NR	NR	NR	1.0	2.6	8.6	8.8	10.0	
	E NR	NR	NR	NR	NR	NR	1.6	3.6	3.8	4.6	
4	C NR	NR	NR	NR	NR	1.6	3.4	6.2	7.0	7.4	
	E NR	NR	NR	NR	NR	NR	NR	0.6	0.6	1.0	
5	C 2.0	4.0	6.6	7.8	10.0						
	E NR	0.4	1.0	1.4	2.0						
6	C 1.4	3.0	3.8	ND	5.0						
	E 0.4	2.4	3.8	ND	4.0						

* Control and experimental segments removed after 4 hours ischaemia.
 ** Dose of Mch in μg/ml bath concentration.

Responses in the experimental strips appeared to be smaller than those in the control strips in every preparation studied. However, the difference was not statistically significant for P = 0.05 but was found to be so for P = 0.1.

TABLE 2.6

IN VITRO RESPONSES OF JEJUNAL STRIPS TO VARIOUS DRUGS*

C = Control, E = Experimental, ↓ = Relaxation, NR = No Response

NaCl x 2 = Double NaCl Ringer Solution, KCl-R = Pure KCl Ringer

Dog No.		Height of contraction in cms			
		D.M.P.P.	Nict.	NaCl x 2	KCl-R
		20 µg/ml	5 µg/ml		
1	C	1.5	NR	2.0	ND
	E	3.0	1.0	2.0	ND
2	C	2.0	1.0	3.5	9.0
	E	1.0	3.5	3.5	8.0
3	C	NR	↓	2.0	8.0
	E	1.0	2.0	2.0	5.0
4	C	NR	NR	NR	6.0
	E	NR	NR	NR	2.0
5	C	1.0	3.5	4.5	12.0
	E	1.0	1.4	3.0	11.0
6	C	2.0	↓	2.2	8.0
	E	NR	NR	2.4	7.5

* No i.a. perfusions had been done on these strips.

this series which had been made ischaemic but had not been previously perfused with i.a. drugs, responded to nicotine and D.M.P.P. in four out of six experiments. In the doses given D.M.P.P. and nicotine were considered to be acting on the ganglion cells. The magnitude of the contractions varied from 1.0-3.0 cms to D.M.P.P. and 1.0-3.5 cm to nicotine. Fig. 2.8 shows the responses of the control and experimental strips to D.M.P.P., nicotine and Double NaCl Krebs Ringer solution. These responses were reduced by hexamethonium 5 $\mu\text{g}/\text{ml}$ and blocked by atropine 0.02 $\mu\text{g}/\text{ml}$. Changing the bath solution to Double NaCl solution produced contraction of approximately the same magnitude 2-3 cms in both the control and experimental strips, in five out of six preparations. Changing to Pure KCl Ringer produced very large contractions (8-9 cms) in both strips. Results to Nic., D.M.P.P. and to changes in the bathing solution are tabulated, Table 2.6. Only the responses to KCl-Ringer were significantly less in experimental segments than in control segments in a paired comparison.

CONCLUSIONS.

The decreased responses to methacholine suggested some degree of damage to the muscle cells in the experimental segment probably due to anoxia. The frequent and sometimes quite considerable responses to D.M.P.P., nicotine and Double NaCl Krebs Ringer solution suggested the presence of some functioning nerve tissue. The next series of experiments were planned to confirm that these responses were indeed due to stimulation of the nerve elements in the intestines.

As in the previous series a segment of whole intestine from the control and experimental areas was saved and fixed in 10% basic formalin and sent to the Pathology Laboratory for histological sections and diagnosis (For results see later).

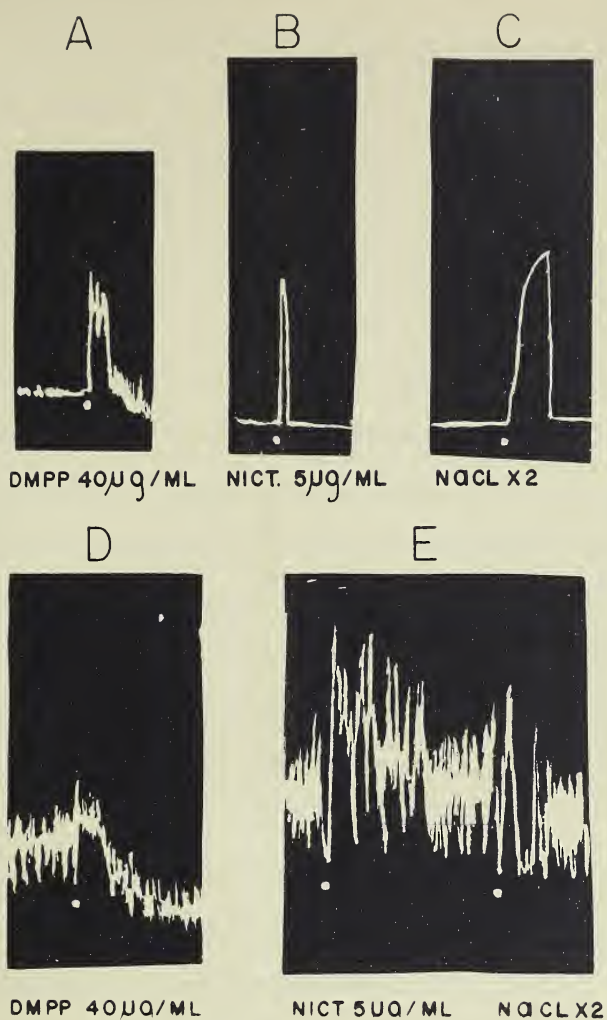


Fig. 2.8 IN VITRO RESPONSES OF THE CONTROL AND EXPERIMENTAL STRIPS OF JEJUNUM TO D.M.P.P., NICT. AND DOUBLE NaCl KREBS RINGER.

A. B. and C. are the response of experimental strips of jejunum.

D. and E. are the responses of the control strip.

III. TRANSMURAL STIMULATION.

The previous series of experiments suggested that functioning neuronal elements were present in the intestinal preparations that had been subjected to 4 hours ischaemia. It therefore became necessary to investigate the reliability of Hukuhara's technique in destroying all functioning ganglion cells and nerve tissue, and hence its ability to effectively produce an aganglionic or completely denervated loop. Since our knowledge of the intrinsic plexus and its function is incomplete, there is no single way by which we could prove whether or not complete destruction of the ganglion cells and their axons had taken place. Microelectrode studies of the intrinsic plexus have never been published. Furthermore, the specificity of the various stimulating and blocking agents is uncertain. Experiments were therefore designed for the following purposes.

1. In acute preparations to stimulate electrically the nervous elements of the intestinal segments by transmural stimulation with varying voltage and frequencies.
2. To localise the site of action of these electrical stimuli by the use of known blocking agents.
3. To get histological evidence of the true condition of the ganglion cells i.e., to determine the number of normal versus damaged ganglion cells, to find out if the damage was reversible or irreversible and to correlate these findings with physiological evidence as to neuronal function. In addition,

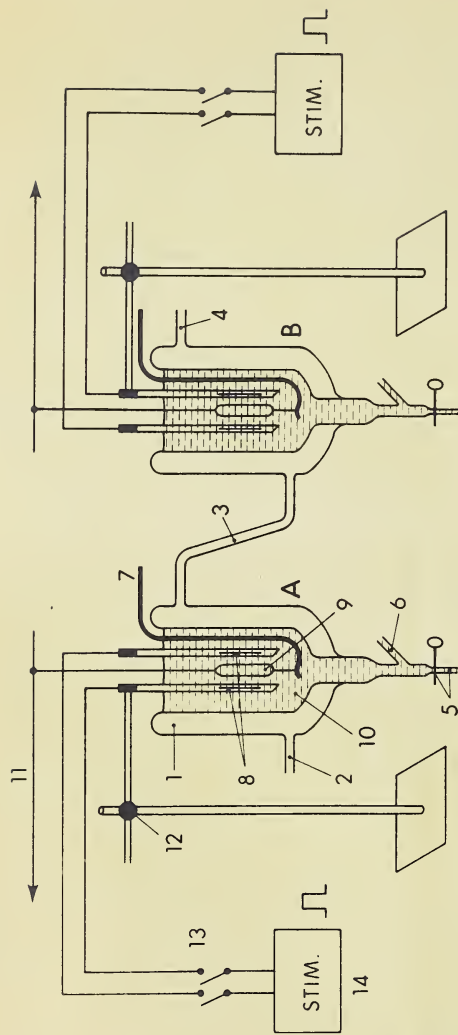


Fig. 2.9 TISSUE BATH AND ORGAN BATH FOR TRANSMURAL STIMULATION

- | | | | |
|------|--|-----|------------------------------|
| A, B | -- tissue baths | 8. | -- platinum plate electrodes |
| 1. | -- water jacket for temperature control | 9. | -- intestinal strip |
| 2. | -- inlet for water (T controlled 37°) | 10. | -- Krebs Ringer solution |
| 3. | -- tubing connecting outlet of tube A to inlet of tube B | 11. | -- writing lever |
| 4. | -- outlet of water jacket | 12. | -- clamp holding electrodes |
| 5. | -- outlet for draining bath and clamp | 13. | -- timer activated |
| 6. | -- inlet for Krebs Ringer solution | 14. | -- stimulator |
| 7. | -- glass suspending hook and O ₂ inlet | | |

chronic experiments were carried out in which the segments were prepared and the dog allowed to live for 10-15 days. This period was expected to be sufficient for the recovery of the muscle cells from acute trauma. Regeneration of nerves would require longer periods. With recovery of the muscle, slow waves, spikes and reflexes could be better studied. In addition transmural stimulation was used to test for the presence of functioning neuronal elements as in the acute experiments and histological preparations were studied. It was hoped that correlation of results from these experiments would show whether ischaemia of 4 hours duration caused anoxia of sufficient degree to produce an aganglionic or a completely denervated loop.

METHODS

A. Acute Experiments. (3)

Surgery. The surgery and the technique for producing ischaemia was similar to that previously described (page 80). The experimental segment was kept ischaemic for 4 hours. A similar procedure was carried out in the control segment but no actual ischaemia was produced.

Preparation of Tissue. 30 minutes after the re-establishment of blood supply the control and experimental segments were removed and immediately placed in oxygenated Krebs Ringer solution. Intestinal strips (0.3 - 0.5 cm x < 3 cms) were prepared and the mucous membrane gently scraped away.

Apparatus. Two organ baths of 150 mls capacity were set up as shown in (Fig. 2.9). The organ baths A and B were filled with Krebs Ringer solution and aerated with 95% O₂ and 5% CO₂ mixture through small glass tubes '7'. The temperature of the

solutions was maintained at 37°C throughout the experiment by outer water jackets '1' through which temperature controlled water circulated. The inner baths '10' were filled and emptied manually by opening the pinchcocks '5' attached to Y-tubes. The lower end of the intestinal strips '9' was attached to small metal hooks on the glass tubes supplying the oxygen. The upper end of the strips was attached by cotton threads to the frontal writing levers '11' which exerted a load of 1 gram. Isotonic contractions of the preparations were recorded with 3 fold magnification on smoked drums.

Stimulation. Each intestinal strip was stimulated between two platinum plate electrodes '9' held at approximately 1.0 cms apart and not touching the tissue. These are placed in the organ bath one on the serosal side and the other on the mucosal side. An electronic stimulator (AEL) (12) was used to deliver monophasic square wave pulses at various frequencies to the platinum plate electrodes and the timer '13' was set to give a stimulus lasting 4 secs., at intervals of 4 minutes. The pulse duration was fixed at 5 msec. throughout the series and chosen as the most appropriate after preliminary experiments. A stimulus strength of 120-150 v delivered at a frequency of 1 pulse/sec. gave minimal responses, and 150 v at a frequency of 10 pulses/sec. provided a submaximal stimulus. (This is the maximal output of the stimulator used and may not be the true maximal stimulus for these tissues).

Drugs Used

Methacholine	-	1 µg/ml and 10 µg/ml.
		(Calculated as its base)
Nicotine	-	200 µg/ml
Hexamethonium Bitartrate	-	100 µg/ml 1 mgm/ml 10 mgm/ml
		(Calculated as its base)
Atropine Sulphate	-	10 µg/ml (Calculated as its base)
Tetrodotoxin	-	100 µg/ml
Pure KCl-Krebs Ringer Solution	-	(as described previously)

(B) Chronic Experiments (11)

These dogs were immunised, de-wormed and kept in isolation for 7-10 days prior to surgery.

Anaesthesia: 6% Nembutal in a dose of 30 mgm/Kg was used for induction and 1/2 cc of the solution was given when required throughout the day.

Fluid Replacement: During the operation this was provided by an intravenous drip of 5% Dextrose in saline 500-750 mls. In four dogs Dextran 6% was given.

Surgery: Surgery was performed under aseptic conditions. All linen, surgical instruments and solutions were autoclaved. The dog's abdomen was cleaned with soap and water and painted with "Ioprep" solution. The abdomen was opened by the usual procedure and the greater omentum gently reflected. Care was taken to handle the intestines very gently and to disturb the surrounding viscera as little as possible while locating the jejunum. All bleeding points occurring in the course of the

surgery was carefully tied. The jejunal segment was taken out of the abdominal incision and packed with warm saline pads.

The control segment was not touched in this operation.

Ischaemia was produced using the same technique described for acute experiments except for the perfusing solution and the method used to flush out the blood.

(1) Non-oxygenated Glucose-free Krebs Ringer Solution and the Stagnant Method. This series will be subsequently referred to as the "Simple Krebs Ringer Series". In this series, sterile, non-oxygenated glucose-free Krebs Ringer solution was used as the perfusing solution. After all the blood had been expelled from the segment, the vessels were left clamped as previously described and the segment kept completely ischaemic for 4 hours.

(2) Non-oxygenated Glucose-free Krebs Ringer Solution and the Constant Perfusion Method. This series will be subsequently referred to as the "Constant Perfusion Series". This method had been used by Szurszewski et al. (130) in conducting similar experiments. In this series, constant perfusion of the segment with non-oxygenated glucose-free Krebs Ringer solution was carried on for 4 hours, using the constant perfusion pump. The rate of perfusion was adjusted at 0.125-0.2 cc/min. and blood such as to prevent a return of venous/ from too slow a rate, or edema of the tissues from too fast a flow.

(3) Non-oxygenated, Glucose-free, Krebs Ringer Solution Saturated with N₂, and the Stagnant Method. In subsequent descriptions this series will be referred to as the "Nitrogen Series". At the end of a perfusion, the Krebs Ringer solution

that was left in the syringe was tested for O_2 with an oxygen electrode "The Beckman Oxygen Sensor". The P_{O_2} of the solution was found to be 12.6×10^{-2} atmospheres which was much higher than expected. To reduce the amount of O_2 absorbed from the atmospheric air, possibly during preparations, the sterile non-oxygenated, glucose-free, Krebs Ringer solution was bubbled with N_2 gas through a sterile porous tube for 1 hour prior to perfusion. The bottle was then capped tightly till it was ready for use. The solution from the bottom of the flask was drawn into a 50 ml sterile syringe through a wide polythene cannula weighted at the tip. With these precautions it was possible to reduce the P_{O_2} of the solution at the end of the perfusion to 1×10^{-2} At. The stagnant method was used and the vessels were left clamped after all the blood from the segment had been flushed out. At the end of the 4 hours the blood supply to the segment was restored and warm saline pads were applied. The cut mesentery was carefully repaired with "000" black silk. All ligatures on small blood vessels were carefully cut and removed to ensure an adequate blood supply to the segment during the period of recovery. The abdominal cavity was cleaned of blood clots and fluid and the segment replaced in the abdominal cavity. Fig. 2.10 (a) and (b) shows the appearance of the segment during ischaemia and just before it was replaced in the abdominal cavity. In one dog a jejunal loop 15 cm long and consisting of a normal and a post-ischaemic area was isolated except for the continuity of the main vessels, and exteriorised. The loop was placed outside the abdominal wall and under the skin. The

Fig. 2.10 (a) A jejunal loop with a segment made completely ischaemic

A,B are the rubber ligatures

a = mesenteric artery with clamp

b = mesenteric vein with clamp

c = arterial branch of vasa recta with cannula

Notice the cut mesentery on either side of the vessels

Fig. 2.10 (b) The jejunal loop 30 minutes after the restoration of blood supply. The sutured mesentery is shown by the black silk thread.

A



B



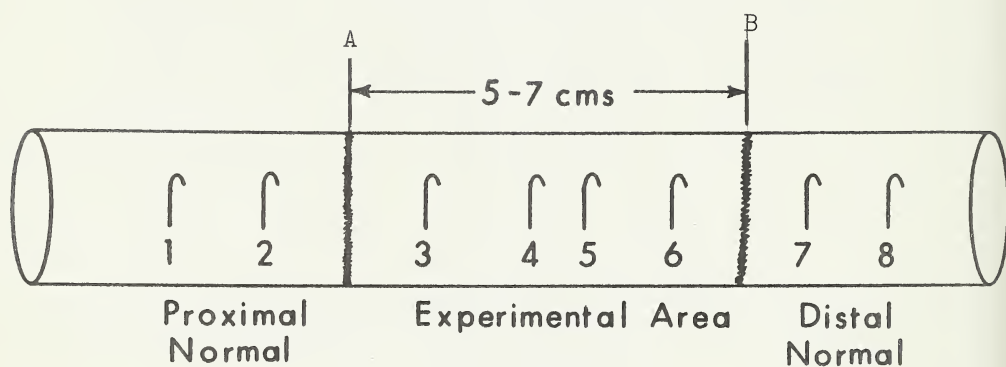


Fig. 2.11

SEGMENT OF JENUNUM

Length of experimental area 5-7 cms. A and B are the borders of normal and experimental areas. Numbers 1-8 are the electrodes under the serosa.

continuity of the bowel was maintained by an end to end anastomosis. Reflexes were tested on the dog on the 21st day.

Postoperative Care: 1. Routine antibiotics were given for 5-6 days postoperative.

2. Dextrose 5% in saline and amino-acids were given parenterally on the next day as a routine and subsequent days in dogs that needed such therapy.

3. Liquid diet in form of milk and clear broth was given for 3 days, changing first to semi-solid pabulum and then to normal meat diet after the 6th day.

Statistics: 18 dogs were operated on. Experiments were performed on 11 good preparations on the 10th - 14th day. In 4 preparations the redistribution of blood supply on unclamping the vessels was poor. The bowel perforated at varying intervals from the 3rd to the 6th day. Postoperative pneumonia and ripping the sutures were the cause of death of the other 3 dogs.

Experimental Procedure (10-15 days postoperative)

10-15 days after surgery the dogs were again anaesthetised with urethane-chloralose mixture and the abdomen opened by a paramedian incision to avoid the scar tissue.

In Vivo Experiments: Electrodes were placed in the experimental segment and adjacent areas as shown. (Fig. 2.11) Electrode 1 and 2 are proximal to the experimental segment, E_{3,4,5,6}, are in the experimental segment and E₇ and 8 are distal to the experimental segment. Electrodes 2 and 3 are equidistant on

either side of the border between the normal and experimental areas. Electrodes 7 and 8 are similarly placed. The configuration frequency and conduction of slow waves and spikes were studied. Effects of i.v. physostigmine and morphine on these activities were also studied.

Response to Drugs: The control segment was prepared and cannulated. Any adhesions in the experimental segments were gently separated at this time and a cannula inserted as in the control segment. Balloons and electrodes were placed as previously described (see Methods and Fig. 2.1) and the responses of the two segments to i.a. perfusion of drugs were studied.

Reflexes: Reflex responses in the segment were studied by injecting 2-3 ccs of 0.1 N HCl into the lumen through a polythene cannula introduced into the lumen together with the balloon. Changes in intraluminal pressure was recorded with pressure transducers (described previously in Section I). Reflex responses to increased intraluminal pressure was also studied by inflating the balloon with 4 ccs of air and releasing till the original pressure (with 1 cc was obtained. In two preparations strain gauge attachment of the type used by Bass et al. (56) was sewn in the longitudinal direction on the serosal surface of the intestinal wall. This provided a more sensitive method of recording contractions but from a more localised area.

In Vitro Experiments (11)

Transmural Stimulation: Electrical stimulation across the intestinal wall was carried out as described for the acute series using the same apparatus Fig. 2.9.

Effects of Blocking Agents on Transmural Stimulation. Effects of hexamethonium 0.1 μg - 10 μg on responses of the strips to transmural stimulation was studied. Effect of atropine 0.02 $\mu\text{g}/\text{ml}$

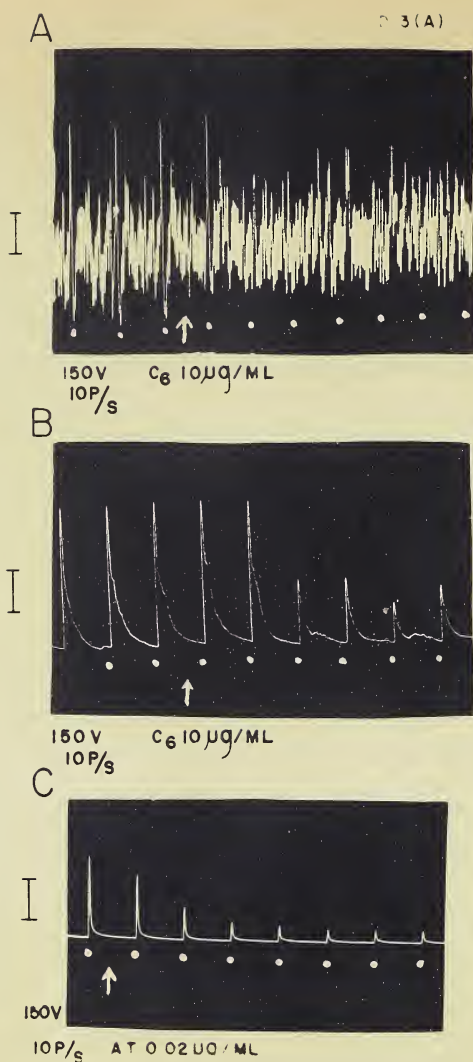


Fig. 2.12. IN VITRO response of the jejunal strip to transmural stimulation. (A) control strip (B) experimental strip. Partial block is achieved with hexamethonium (C_6) 10 μ g/ml (C) Experimental strip and complete block of residual response with atropine 0.02 μ g/ml

on these responses was also studied. Methacholine 0.005 $\mu\text{g/ml}$ and Pure KCl-Krebs Ringer solution in the bathing medium was used to test the integrity of the muscle.

Tetrodotoxin: Effect of tetrodotoxin 0.1 $\mu\text{g/ml}$ and 0.5 $\mu\text{g/ml}$ on the response to transmural stimulation and on responses to D.M.P.P. and Nict. was also investigated.

RESULTS

(A) Acute Dogs (3)

The experiments were performed on the same day as the surgical procedure for producing ischaemia and no i.a. perfusions had been given.

Pendular Movements: These were not seen in any of the experimental strips in this series of experiments. Control strips showed pendular movements 15-30 mts after suspension in the organ bath.

Transmural Stimulation

In this series responses in the experimental strip were seen only when stimulus of 150 v was given at 10 pulses/sec. The pulse duration was fixed at 5 msc. The strips responded with contractions of 2-4 cm. height in the experimental strip and 3-8 cm height in the control strip.

Effect of Blocking Agents: (a) Hexamethonium 5-10 $\mu\text{g/ml}$ reduced the height of contraction produced by electrical stimulation to 50-75% in both the control and the experimental segments. Fig. 2.12 (a) and (b). Nicotine 5 $\mu\text{g/ml}$ added to the bath containing 5-10 $\mu\text{g/ml}$ hexamethonium produced a relaxation in both strips. After 10 $\mu\text{g/ml}$ hexamethonium responses to Mch were also reduced. (b) Atropine 0.02 $\mu\text{g/ml}$ completely abolished

the residual contraction to electrical stimulation that had not been blocked by hexamethonium in both strips. Fig. 2.12 (c). Pendulum movements in the control were also abolished.

Tetrodotoxin: 0.1 $\mu\text{g/ml}$ and 0.5 $\mu\text{g/ml}$ was tested on one preparation. In other intestinal preparations reported in literature, such doses of tetrodotoxin abolished responses dependent on neurones, but had no effect on responses independent of neurones (139,140). In the jejunal strips studied tetrodotoxin completely abolished responses to electrical stimulation in both the control and experimental strips, indicating that the structures stimulated were nerve elements in the intestines. Tetrodotoxin had no clear effect on the responses of the strips to Mch and Pure KCl Krebs Ringer solution (Later in chronic dogs, this was found to be also slightly reduced). Tetrodotoxin 0.1 $\mu\text{g/ml}$ did not abolish the responses to D.M.P.P., nicotine and Double NaCl Krebs Ringer solution in either the control or the experimental strips, although the height of the contractions was reduced. Tetrodotoxin 0.5 $\mu\text{g/ml}$ completely blocked the responses of the strips to D.M.P.P., nicotine and Double NaCl Krebs Ringer solution. Responses to Mch and KCl were not affected and even appeared to be potentiated.

CONCLUSIONS

1. The jejunal strips of both the control and the experimental segments after 4 hours ischaemia could be electrically stimulated to contract.
2. These contractions were probably due to stimulation of both pre- and postganglionic cholinergic nerves. This is indicated by the ability of hexamethonium to partially block these responses and atropine to completely prevent them.

3. Tetrodotoxin which had been shown to abolish nerve action potentials by affecting Na conductance, leaving smooth muscle action potentials unaffected, abolishes the responses of the strips to transmural stimulation. It does not affect the responses to Mch. This gives added evidence that the contractions produced by transmural stimulation was due to action of the stimuli on functioning nerves in the intestinal wall.

(B). CHRONIC DOGS

In Vivo Experiments (11)

Appearance of the Segment: On opening the abdomen, the segment appeared normal. Some adhesions were present around the segment, and in the adjoining mesentery but could be easily teased away or cut between ligatures. The experimental segment was identified by two slightly constricted and pale areas where the ligatures had been applied in the first surgery. Black silk used for suturing the mesentery also served as a guide. In 3 dogs the experimental area was slightly constricted but in the others they were normal or appeared to have less tone. Small patches of healed tissue covered with dense adhesions were seen in 4 dogs; probably where a small perforation or ulcer had healed. These appeared at the ends of the segment having the smallest arteries and probably the poorest blood supply. These adhesions were gently cut away and did not interfere with the recording or with the responses. Sluggish rhythmic contractions were seen in all 6 dogs.

The Electrical Activity: Slow waves were more regular in the experimental segments of chronic preparations than in those of acute dogs. The configuration of the slow waves in the

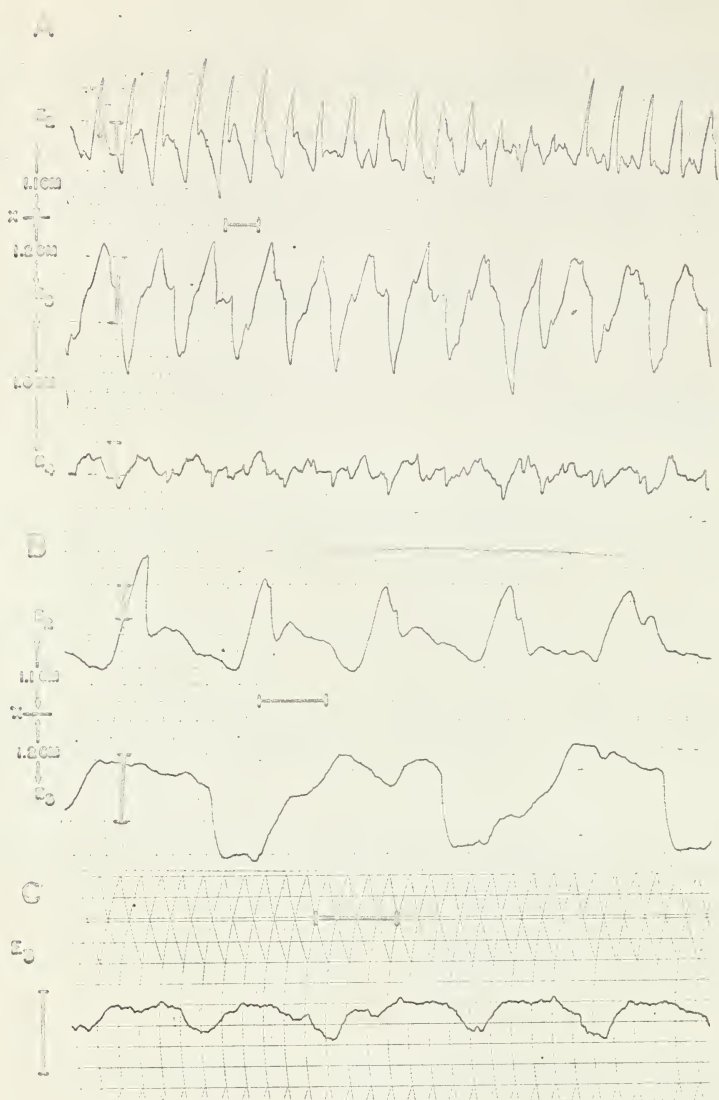


Fig. 2.13. Slow waves from the jejunum, recorded IN VIVO with electrode E₂ in the proximal normal area E₃ and E₄ in the experimental area (see Methods). Distance between electrodes are as shown and 'x' marks the boundary between the normal and the experimental areas. Notice small and irregular slow waves in E₄, lower frequency of the slow waves and marked change in configuration in E₃ (A, B & C). (B & C) are at greater paper speed. This was from the Nitrogen Series. Vertical marking denotes 1 mv; the horizontal marking - 4 sec.

experimental area did not always conform to the common pattern in size or shape. Their amplitudes were low (0.5 - 1.5 mv) with a slower rate of positive deflection and either a sharp fall or a slow decline to the original level Fig. 2.13 (b) and (c).

Eight electrodes had been placed in the jejunal loop (Fig. 2.11, page 108), E1 and E2 which were proximal and outside the experimental area recorded slow waves of normal size and shape. E3, in the experimental area and about 1.0 cm inside the boundary recorded slow waves of 0.5 - 1.5 mv. There was usually a marked change in the shape of the slow waves in E3 compared to E1 and E2, Figs. 2.13 and 2.14. In preparations where the stagnant method had been used to keep the segment ischaemic (see Methods, page 106) regardless of whether non-oxygenated, glucose-free Krebs Ringer solution or the N_2 bubbled solution was used the amplitudes of the slow waves decreased with increasing distance of the recording electrodes from the proximal boundary. E4 or E5, usually in the centre of the experimental area recorded very small slow waves and were usually irregular Fig. 2.14. Electrodes nearer the lower boundary of the experimental segment (E6 was approximately 10 cm away from the boundary) the slow wave amplitude became larger and approached the shape and size recorded in E7 and E8 distal and outside the experimental area. No spikes were recorded from electrodes in the experimental area in any of the preparations carried out with this method.

In preparations where constant perfusion had been carried on throughout the 4 hours, the slow waves were regular in appearance and were of approximately the same amplitude in all electrodes in the experimental area. They were however

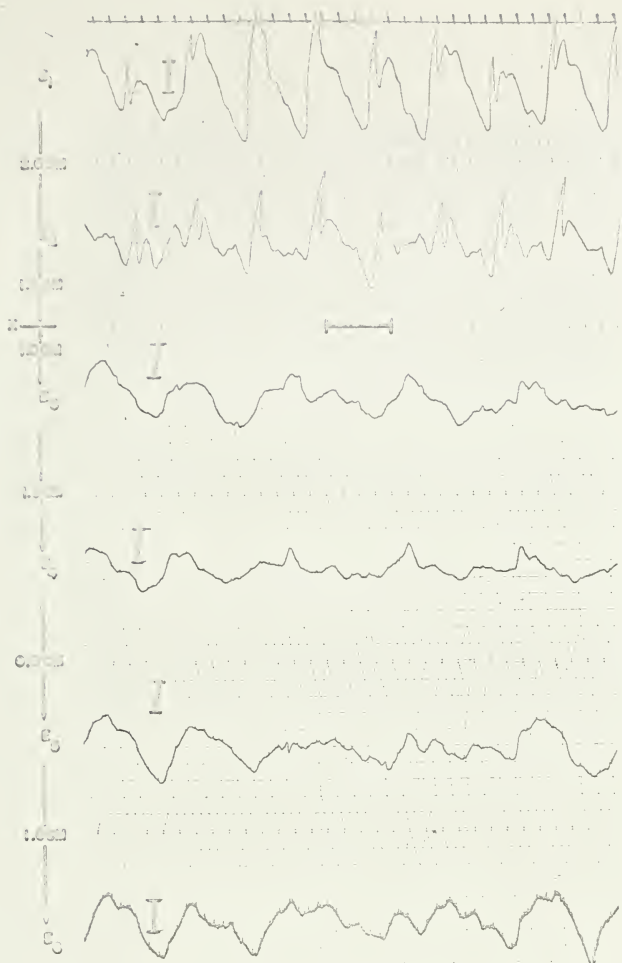


Fig. 2.14. S.W. in the jejunum recorded IN VIVO with electrodes in the normal and experimental area at distances marked. E_1 and E_2 are in the proximal normal area E_3 - E_6 are in the experimental area. 'x' is the boundary between the normal and experimental area. Notice the decrease in amplitude with increasing distance from 'x'. Amplitude gets larger (E_7) as the recording electrode gets nearer E_7 and the lower border. This was from the N_2 series. The vertical line denotes 1 mv and the horizontal line 4 sec.

TABLE 2.7

FREQUENCIES OF SLOW WAVES AT DIFFERENT ELECTRODES IN THE JEJUNUM

Distance between the electrodes was roughly 1.5 to 2.5 cms. E1 and E2 are in the area proximal to the experimental segment. E3, E4, E5 and E6 are in the experimental segment and E7 and E8 are in the area below the experimental segment.

Experiment No.	Frequencies recorded at different electrodes E.							
	E1	E2	E3	E4	E5	E6	E7	E8
1	15	15	*	*	*	15	15	15
**								
4	16	16	10	9	*	13	13	13
5	15	15	11	11	11	12	12	12
6	14	14	12	10	10	11	12	12
7	15	15	12	12	8	10	11	11

9	16	16	9	9	12	12	14	14
10	16	16	9	9	9	10	10	10
**								

* Adhesions in the area and no electrodes inserted.
** No in vivo experiments were carried out in experiments 2, 3, 11.
*** In experiment No. 8, the loop was exteriorised. Frequency in both normal and experimental were low, 12/min and 8/min respectively.
Difference in frequency of slow waves between the proximal normal area (E1 and E2) and that in the postischaemic area (E4 and E5) was found to be statistically significant at $P \leq 0.001$. Similarly the frequency of the slow waves in the distal normal area (E7 and E8) was significantly different from the frequency in the upper proximal area (E1 and E2) at $P = .02$ and from that in the postischaemic area (E4 and E5) at $P = .01$.

smaller than those recorded in E1 and were of a different shape, again with a slow rate of rise and a sharp fall. A few spikes were recorded from time to time. Fig.2.15 in E5 and E6. (arrows) Electrical records performed by the perfusion method are shown in Fig. 2.15.

Frequency: From E1 and E2 in the normal area slow waves had a frequency of 14-16/min. The frequency of slow waves in the experimental area was lower at all the electrodes, irrespective of the solution and method used to make the segment ischaemic. They ranged from 9-12/min. In the distal normal areas, E7 and E8 had frequencies of 11-14/min.; a higher rate than in the experimental area but lower than in the proximal normal area. The distance between slow waves, peaks and troughs in the E2 (control) and E3-6 (experimental) are shown in Fig. 2.16. The frequencies of the slow waves recorded at different electrodes from 9 dogs is given in Table 2.7.

Conduction of Slow Waves: Study of conduction of slow waves in the experimental area was difficult because of the low amplitude and the irregularity of the slow waves in E4 and/or E5. In 2 experiments carried out with constant perfusion, the slow waves were larger, appeared more regularly and were definitely conducted within the area. This is more apparent after an i.v. injection of morphine 200 μ g/Kg. (Fig. 2.17). Conduction between the normal and the experimental segment either from above or below was not often seen though slow spread sometimes occurred to E3 and E6. In the proximal normal area, propagation occurred aborally from E1 to E2. In the distal normal area, propagation was often orad and occurred from E8 to E7, Fig. 2.18.

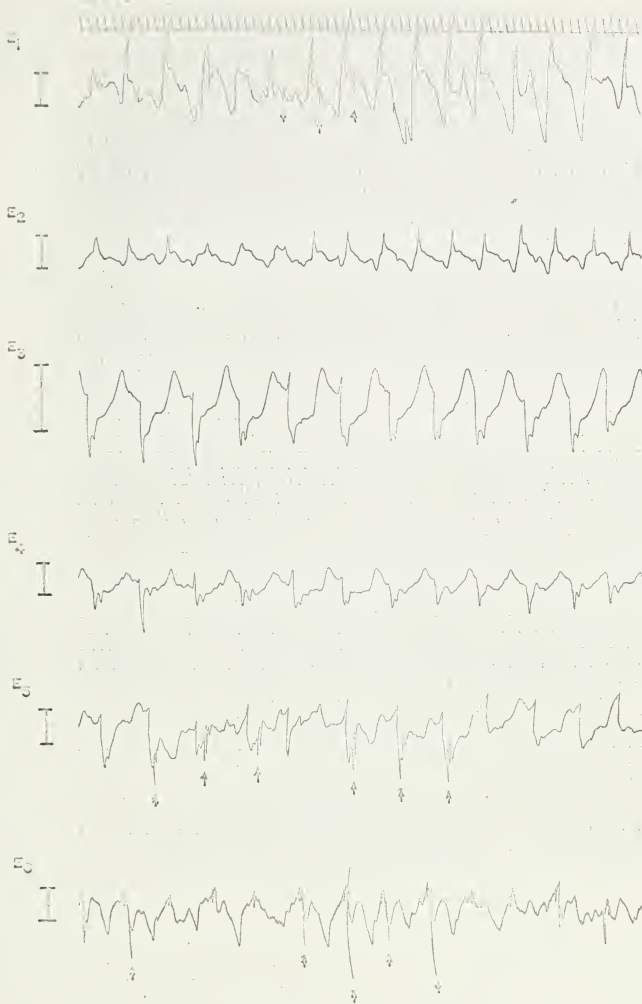


Fig. 2.15. Electrical activity from the jejunal segment IN VIVO with electrodes placed at distances shown. E₁ and E₂ are in the normal proximal area and E₃ to E₆ are in the experimental area. Ischaemia was produced by constant perfusion of NOGFKR solution (see Methods). Notice the spikes in normal as well as the perfused area (arrows). Vertical line denotes 1 mv, horizontal line-4 sec.

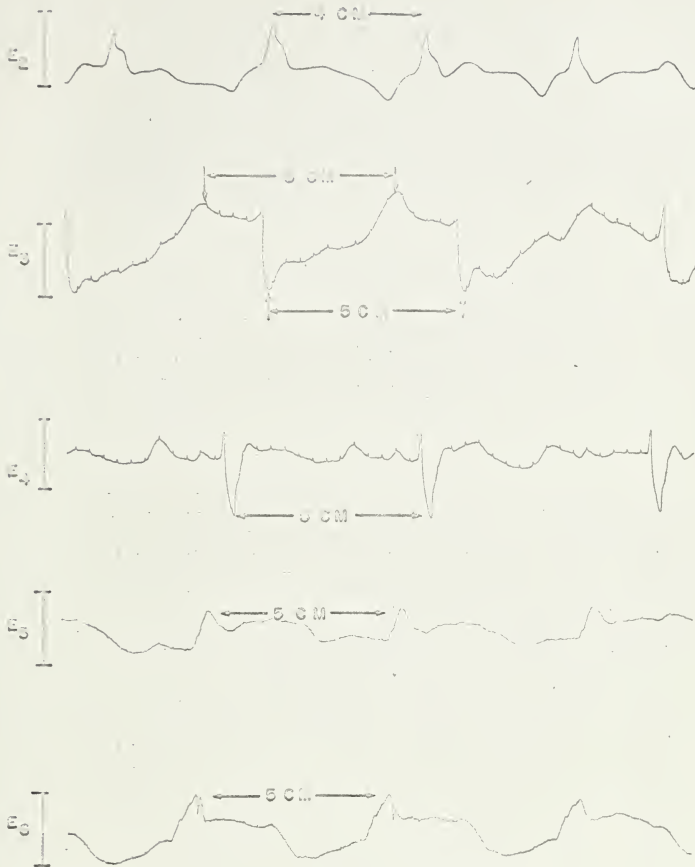


Fig. 2.16. Electrical Activity from the jejunal segment of the N_2 series. Distance between slow wave peaks and troughs in the control (E_2) and experimental areas. (E_3 - E_6). There is a greater distance between slow wave peaks, consequent of a slower frequency.



Fig. 2.17. Conduction of slow waves in the experimental segment after morphine 200 $\mu\text{g}/\text{kg}$. Ischaemia produced by the perfusion method. Notice the spikes in all electrodes.



Fig. 2.18. Conduction of slow waves in the proximal normal and distal normal areas of the jejunum separated by the experimental area. Notice the conduction in the proximal area is from E_1 to E_2 (dotted line and slow wave displacement) and in the distal area in the opposite direction from E_8 to E_7 .

TABLE 2.8

RESPONSES OF THE CONTROL AND EXPERIMENTAL JEJUNAL SEGMENTS TO DRUGS*

C = Control, E = Experimental, NOGFK-R Sol. = Non-oxygenated Glucose-free Krebs Ringer Solution, ND = Not Done, NR = No Response, SP = Spikes, ↓ = Relaxation (-) Absent (+) Present.

Experiment No.	Solution and Perfusion Method	H ₂ O	P.D.G. 10 µg mm	Nict. 5 µg mm	D.M.P.P. 5 µg mm	Mich. 0.1 µg mm
3	NOGFK-R Sol. Stagnant Method	C	75.0	75.0	ND	87.5
		E	NR	37.5 SP(-)		62.5
5	NOGFK-R Sol. Stagnant Method	C	↓	↓	ND	100.0
		E	↓	↓		75.0
6	Constant Perfusion	C	87.5	87.5	75.0	112.5
		E	75.0	75.0	37.5	87.5
9	NOGFK-R Sol. Saturated with N ₂	C	100.0 SP(+)	87.5 SP(+)	75.0	87.5 SP(+)
		E	75.0 SP(+)	75.0 SP(+)	25.0	75.0 SP(-)
10	Stagnant Method	C	75.0 SP(+)	60.0 SP(+)	87.5	50.0 SP(+)
		E	62.5 SP(+)	37.5 SP(+)	NR	50.0 SP(+)

* Experiments were performed with i.a. perfusion of modified Krebs Ringer solution using different perfusion methods. Drugs were also administered i.a. in 1 ml. volume and flushed with 0.5 ml Krebs Ringer solution. Except for Mch difference in responses between control and experimental segments was not statistically significant at P = 0.05.

These patterns of conduction probably account for the variations in slow wave frequency between the ends of the ischaemic segment.

Effect of Morphine or Physostigmine on Electrical Activity: I.V.

injection of physostigmine 100-200 $\mu\text{g}/\text{Kg}$. or morphine 100 $\mu\text{g}/\text{Kg}$. was given to nine dogs. Five out of nine dogs responded with an increase in amplitude of the slow waves which also became more regular. Morphine was more effective than physostigmine in this respect. There were no spikes in these preparations before injection of the drugs but varying number of spikes appeared 5-10 minutes after injection and lasted for 30 minutes. In two dogs, no response to either physostigmine or morphine was seen. In the other two dogs, constant perfusion had been used to keep the segment ischaemic. The slow waves in these preparations were regular, with few spikes before the drugs were given and no difference in the slow waves could be detected after the drugs, but the number of spikes increased markedly.

Responses to I.A. Perfusion of Drugs: 5 dogs were given i.a.

perfusions of P.D.G., Mch., D.M.P.P. and Nict. to test the responses of the control and experimental segments in chronically prepared dogs. One dog was chosen from the simple Krebs Ringer series (see Methods). Two dogs were chosen from the constant perfusion series and two from the N2 series. Results of these experiments are shown in Table 2.8.

Phenyldiguanide: 10 μg of P.D.G. i.a. resulted in a contraction of 3-4 cms in the control segment in four preparations. One preparation from the perfusion series responded with relaxation, in both the control and the experimental segments. In other experimental segments, there was a contraction of 2-3 cms in

three preparations. Spikes were present in the electrical records of both the control and experimental segments Fig. 2.19.

Responses to Nict. and D.M.P.P.: Response to nicotine was present in both the control and experimental segments in all five dogs. There was a contraction of 3-3.5 cm in the control and 1.5-3.0 cm in the experimental segments Fig. 2.20. Relaxation preceded contraction in one of the preparations. Only relaxation was seen in both the control and experimental segments in the dog in which P.D.G. had a relaxant effect (see Table 2.8). Spikes accompanied the contraction in three preparations. D.M.P.P. was given 30 minutes before Nict. A contraction of 3.0 cm in the control segment was seen in three preparations in which it was used, Fig. 2.21 shows one such response. Contractions of 1.0 cm and 1.5 cm were seen in two of the experimental preparations and no response was seen in one preparation.

Methacholine: Equal doses of methacholine 0.1 μ g were given i.a. in the control and experimental segments in all five dogs. Contraction of 2.0 - 4.5 cms were observed in the control segments and slightly less (2.0 - 3.0 cms) in the experimental segments Fig. 2.22. Spikes accompanied the contractions in all five preparations. Responses to drugs appeared to be approximately the same in all five preparations, irrespective of the solution and the method used for keeping the segment ischaemic.

Reflex Responses: One dog from the simple Krebs Ringer series, one dog from the perfusion series and three dogs from the N2 series were tested for reflex responses. Introduction of 2-3 ccs of 1% 5-HT or 0.1 N HCl into the lumen produced contractions in the control segments. The contractions appeared in about

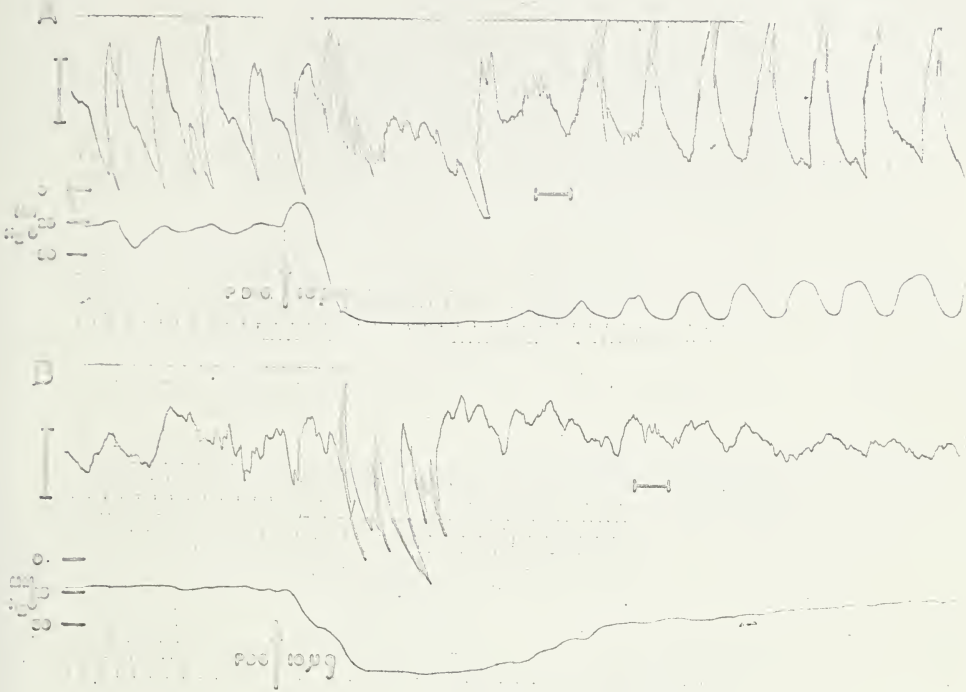


Fig. 2.19. Response of the jejunal segments IN VIVO to i.a. perfusions of P.D.G. 10 μ g at arrows (A) in the control segment and (B) in the experimental segment. Notice the spikes in the electrical record. Vertical marking denotes 1mV and the horizontal marking=4 secs.

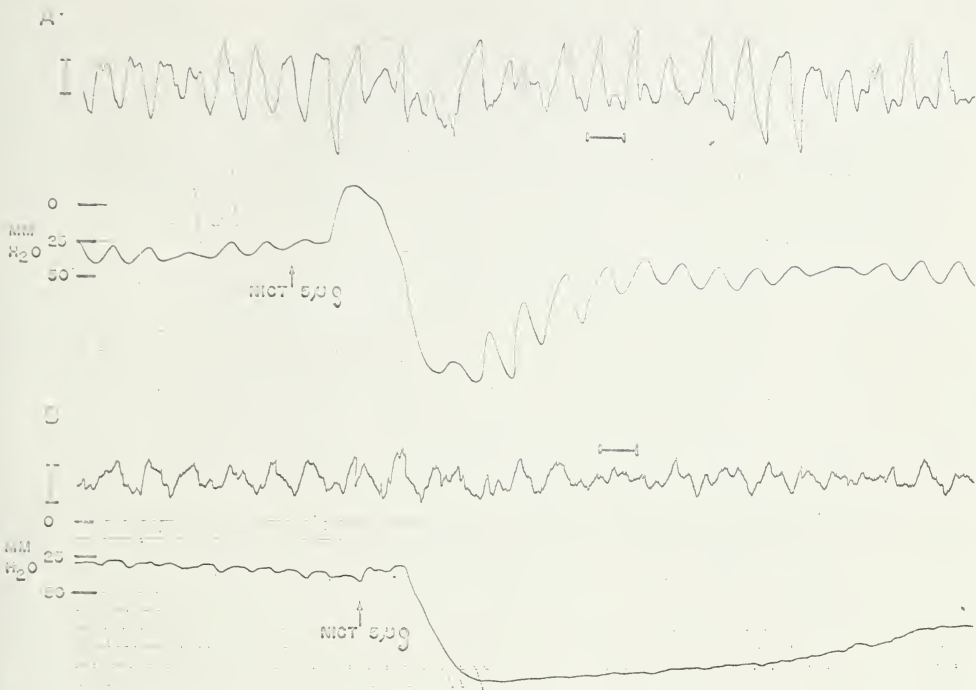


Fig. 2.20. Response of the jejunal segments *IN VIVO* to i.a. perfusion of Nict. 5 μ g at arrows (A) in the control segment and (B) in the experimental segment. Vertical marking denotes 1 mm, the horizontal marking 4 sec.

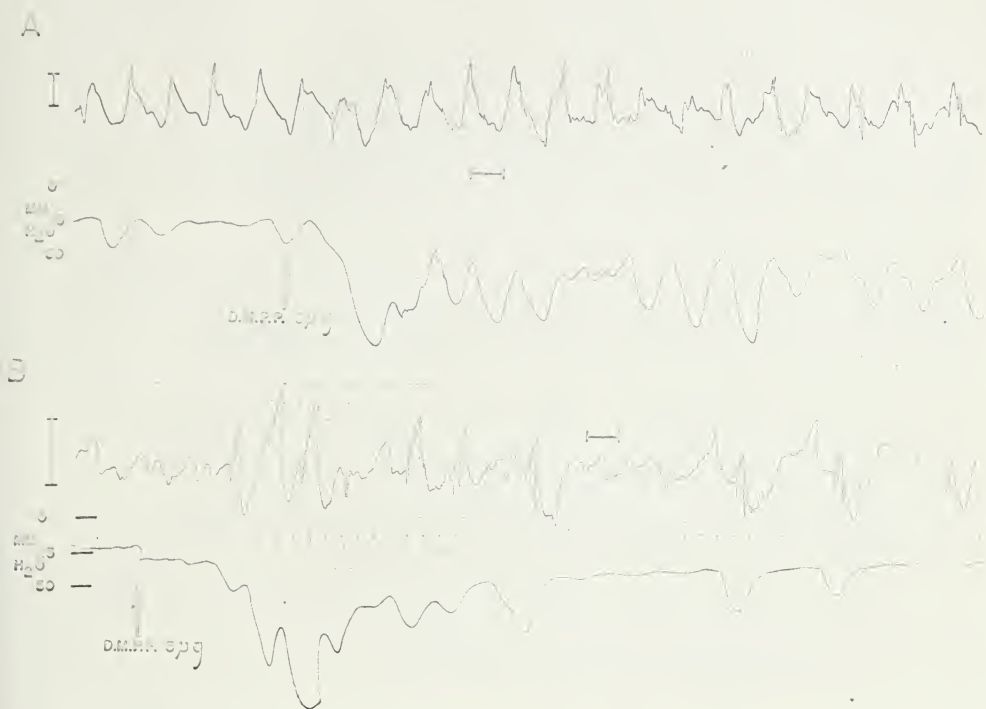


Fig. 2.21. Response of the jejunal segments IN VIVO to i.a. perfusions of D.M.P.P. 5 μ g at arrows (A) in the control segment (B) the experimental segment. Vertical marking denotes 1 mv and horizontal marking 4 secs.

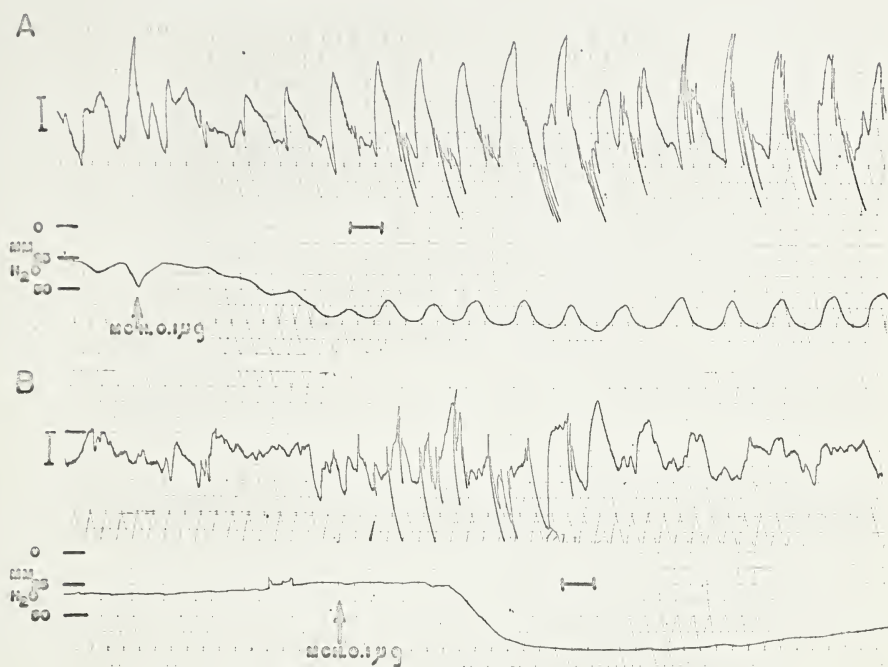


Fig. 2.22. Response of the jejunal segments IN VIVO to i.a. perfusion Mch 0.1 μ g at arrows (A) in the control segment and (B) the experimental segment. Vertical marking denotes 1 mv and horizontal marking 4 secs.



Fig. 2.23 Reflex responses in the jejunum tested IN VIVO with intra luminal application of 0.1 N HCl and increase intra luminal pressure (A) control segment and intra luminal application of 0.1 N HCl (B) control segment and increased intra luminal pressure. (C) Experimental segment and intra luminal application of 0.1 N HCl and (D) Experimental segment and increased intra luminal pressure. Vertical marking denotes 1 mv and horizontal marking denotes 4 secs.

5-10 secs and lasted about 1-2 minutes. Variable number of spikes were recorded in the electrical record. No such responses were observed in any of the experimental segments. Sudden increase of intraluminal pressure, as when the balloon was inflated with 4 ccs of air induced contractions which were recorded better when the distension was reduced (1 cc). No such response was seen in the experimental segments Fig. 2.23. Recording with extraluminal strain gauge attachments gave the same results. (See Methods)

In Vitro Experiments.

Responses to Mch, Nict., D.M.P.P. and transmural stimulation were studied in control and experimental jejunal strips from all 11 chronically prepared dogs.

Spontaneous Activity: In the control strips, pendulum movements were observed in 10 out of the 11 strips studied. No movements were seen in the eleventh strip and responses to drugs and electrical stimulation in this preparation was also very poor. No pendulum movements as such were seen in the experimental strips in 9 out of the 11 preparations studied. The two strips prepared from the perfusion series showed pendulum movements but of a lesser magnitude than the control strips. Spontaneous activity in the form of slow, rhythmic contractions were recorded.

Transmural Stimulation.

Electrical stimulation of control and experimental strips was performed in all 11 preparations (see Methods).

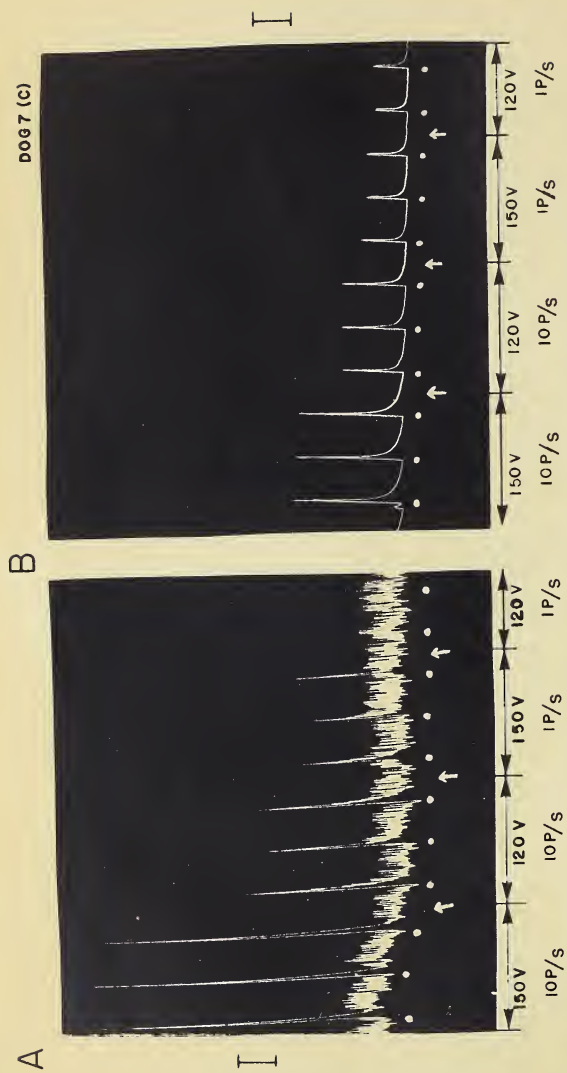


Fig. 2.24. Response of jejunal strips from chronic dog to transmural stimulation IN VITRO with varying voltage and frequency.
 (A) control strip (B) experimental strip.
 Notice the pendular movements in (A) and its absence in (B).
 The marking at the side = 1 cm.

Varying the Strength of the Stimuli.

Stimuli of 120 v - 150 v were delivered at 10 pulses/sec. for the high frequency stimulus and 120 v - 150 v delivered at 1 pulse/sec. provided the low frequency stimulus. Pulse duration was 5 msec. in all cases. The low frequency stimulus produced responses in the experimental strips of between 1.0-3.0 cms. Contractions in the control segments at such stimulus strengths were not detectable because of marked pendulum movements present in these strips Fig. 2.24. Increasing the frequency to 10 pulse/sec. using the same voltages produced detectable contractions which were now larger than the pendulum movements. The height of these contractions varied from 4.5 - 9 cms in most preparations. In only 2 preparations were they below 4.0 cms. The contractions in the experimental strips were also proportionately higher and varied from 3.0 - 9 cms. The type of contractile responses obtained by transmural stimulation with different stimuli strength is shown in Fig. 2.24. Due to the various factors involved such as the size of the strip, and the distance of the 2 electrodes from the tissue and from each other, the voltage current recorded at the stimulator output does not indicate the amount of current passing through the tissue.

Response to Drugs.

Responses to Mch, D.M.P.P. and Nict. were tested before addition of the blocking agents (hexamethonium and atropine) and after the responses to transmural stimulation is reduced or abolished.

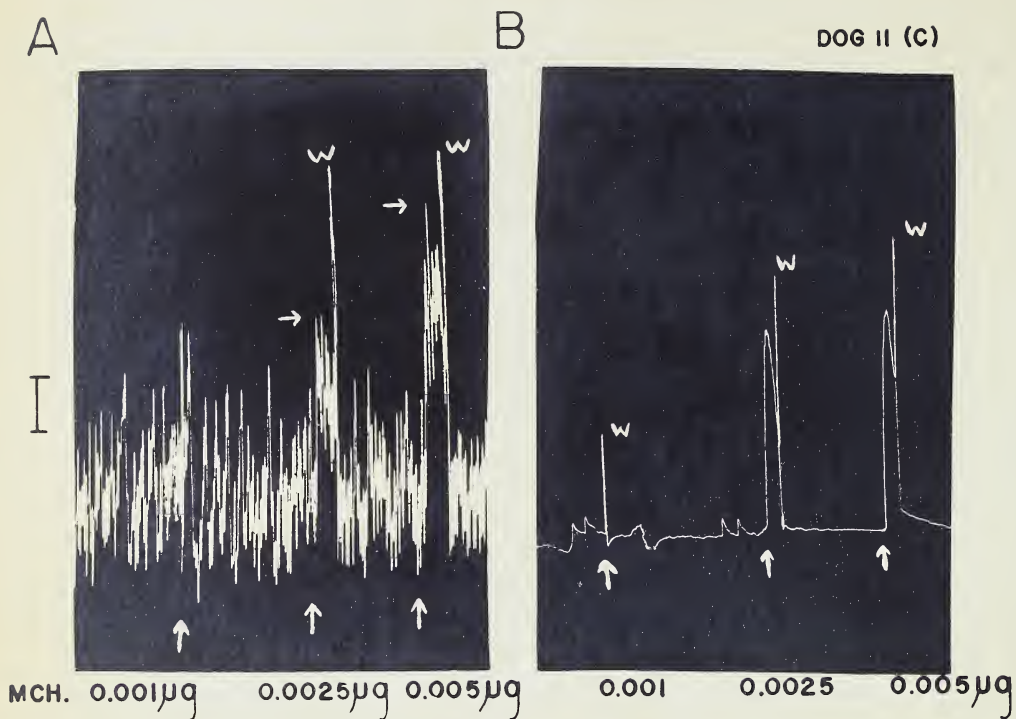


Fig. 2.25. Response to methacholine IN VITRO of the jejunal strips from (A) control and (B) experimental strips from segments of chronically prepared dogs. The marking at the side = 1 cm.

Methacholine: The isotonic responses to Mch 0.001 μg and 0.005 μg were tested. The range of the lever excursions during responses were lower in the experimental strips (2-8 cms) than in the control strips (3-9 cms), but in many instances the responses produced by 0.005 $\mu\text{g}/\text{ml}$ were approximately the same Fig. 2.25.

Nict. and D.M.P.P.: Nicotine was tested on eight preparations and D.M.P.P. on three preparations. Nicotine 1 $\mu\text{g}/\text{ml}$ induced contractions and produced lever excursions of 1.0 - 3.5 cms in the control strips of six preparations, followed by a long period of relaxation during which there were no pendulum movements. Responses to 150 v and 10 pulse/sec were also reduced and those to lower voltage and frequency abolished in both the control and experimental strips Fig. 2.26. The relaxation responses to Nict. in the experimental strips were less than in the control strips but contractions were approximately the same in both and was present in all six preparations. Two preparations gave contractions as high as <3.0 and 3.0 cms in both the experimental and control strips Fig. 2.27. These contractions were not always followed by relaxations. No response to nicotine was seen in two preparations. D.M.P.P. 40 $\mu\text{g}/\text{ml}$ produced a contraction of 2.0-3.0 cms in two out of three preparations tested Fig. 2.28.

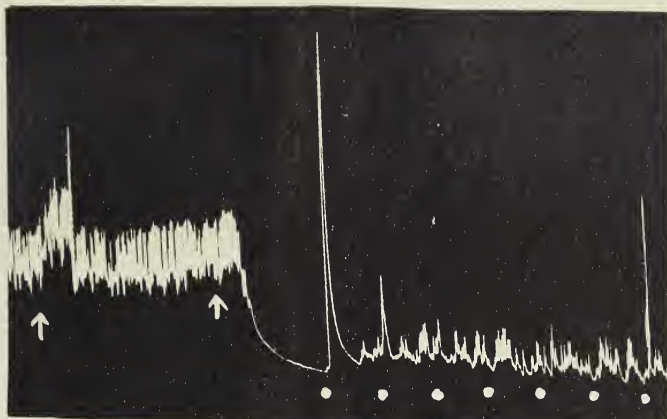
Effect of Blocking Agents on Responses to Transmural Stimulation.

(a) Hexamethonium was added to the bath to give final concentrations from 0.1 $\mu\text{g}/\text{ml}$ - 10 $\mu\text{g}/\text{ml}$. Effect of the ganglion blocking agent on contractions of the jejunal strips to transmural stimulation was studied. Results of these experiments were given in Tables 2.9 and 2.10. Concentrations of 1.0 $\mu\text{g}/\text{ml}$ - 1.0 $\mu\text{g}/\text{ml}$ diminished the height of contractions

A

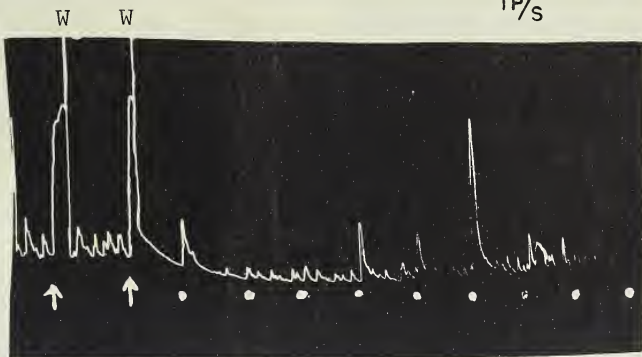
W

DOG 6 (C)



MCH 0.005 $\mu\text{g}/\text{ML}$ NICT 1 $\mu\text{g}/\text{ML}$ 150V 120V 120V 120V 120V 10P/s 10P/s 10P/s 10P/s

B



MCH 0.005 $\mu\text{g}/\text{ML}$ NICT 1 $\mu\text{g}/\text{ML}$ 120V 120V 150V 120V 10P/s 10P/s 10P/s 10P/s

Fig. 2.26. Responses to Mch 0.005 $\mu\text{g}/\text{ml}$ and Nict. 1 $\mu\text{g}/\text{ml}$ in the (A) control and (B) experimental strips from chronically prepared dogs. Notice Nict. 1 $\mu\text{g}/\text{ml}$ blocked responses to low frequency stimulus but does not block responses to high frequency stimulus.

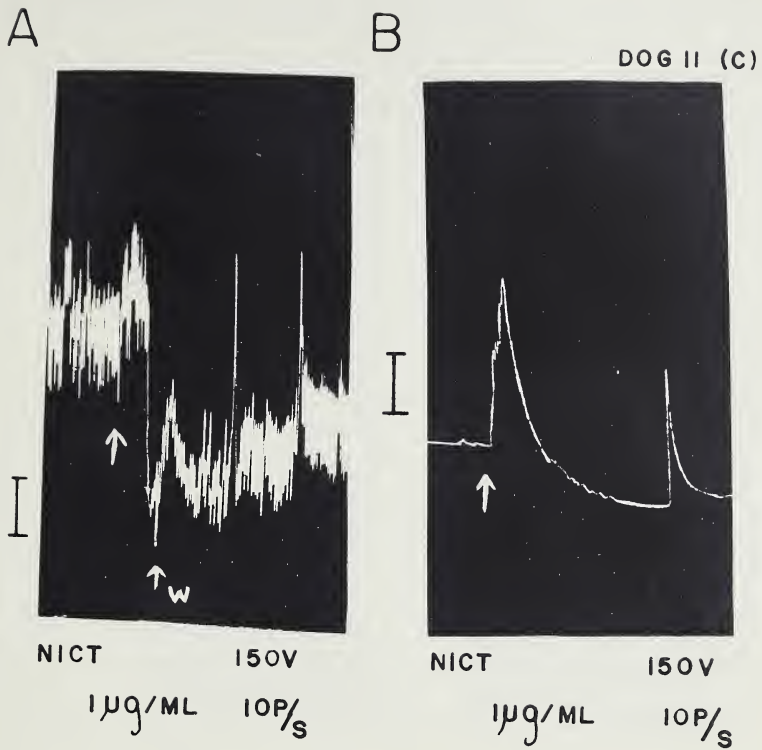


Fig. 2.27. Responses IN VITRO to Nict. $1 \mu\text{g/ml}$ in the (A) control (B) experimental strips from chronically prepared dogs. Notice the response to high frequency stimuli during the relaxation in both strips. The marking at the side = 1cm .

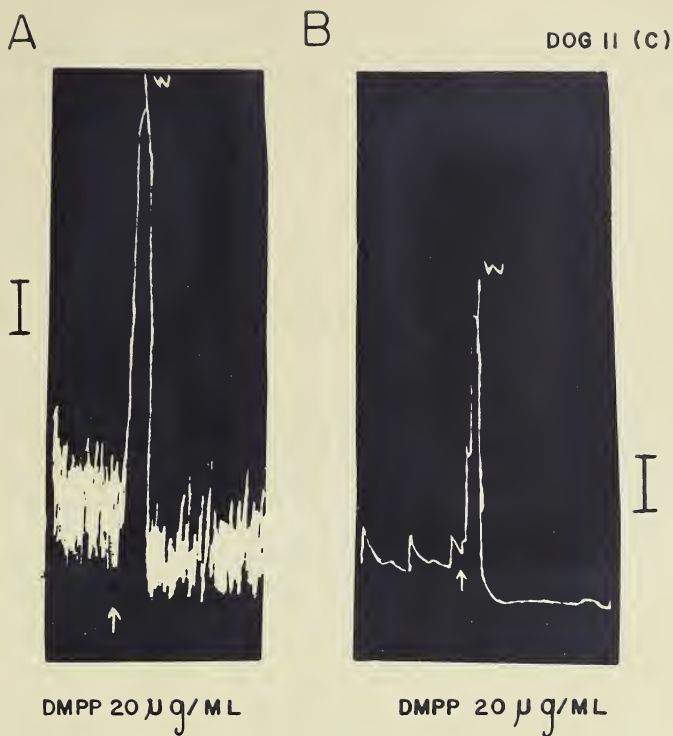


Fig. 2.28. Responses IN VITRO to D.M.P.P. 20 μ g/ml in the (A) control and (B) experimental strips from chronically prepared dogs. (Both records have been touched.) Marking at the sides = 1 cm.

TABLE 2.9

EFFECT OF HEXAMETHONIUM ON RESPONSES TO
TRANSMURAL STIMULATION OF THE JEJUNUM

Effect of hexamethonium (C_6) 0.1 - 1.0 $\mu\text{g/ml}$ on the responses of the jejunal strips to transmural stimulation with 120 v and 1 pulse/sec.

C = Control, E = Experimental, NB = Not Blocked, NR = No Response,
PM = Pendulum Movements (+) present (-) absent

Experiment No.		Height of Contraction in cm.	After C_6	
			0.1	1.0 $\mu\text{g/ml}$
2	C	Uncertain		
		PM +		
	E	4.5		NR
3	C	Uncertain		
		PM +		
	E	1.0		NR
7	C	Uncertain		
		PM +		
	E	1.0		NB Blocked by 5 $\mu\text{g/ml}$
8	C	Uncertain		
		PM +		
	E	1.2		NB
10	C	Uncertain		
	E	2.0		NB

TABLE 2.10

EFFECT OF HEXAMETHONIUM ON RESPONSES OF JEJUNAL STRIPS TO TRANSMURAL STIMULATION

Transmural stimulation of the jejunal strips with 150 v and 10 pulse/sec. after the addition of hexamethonium to the bath to obtain a final concentration of 5-10 $\mu\text{g/ml}$.

C = Control, E = Experimental, Expt. = Experiment, NB = Not Blocked, NR = No Response

Experiment No.	Height of the contraction in mm H_2O			
	At onset of Expt.	After C_6 1-5 $\mu\text{g/ml}$	After C_6 10 $\mu\text{g/ml}$	After At. 0.02 $\mu\text{g/ml}$
1	C 3.0	3.0	1.5	NR
	E 1.5	1.5	1.0	NR
2	C 7.0	4.5	3.0	NR
	E 9.0	9.0	2.5	NR
3	C 4.5	3.5	2.5	NR
	E 7.0	4.5	3.5	NR
5	C 9.0	5.0	5.5	NR
	E 6.5	NR	NR	NR
6	C 6.0	3.5	2.5	NR
	E 5.0	5.5	3.3	NR
7	C 8.0	7.0	3.0	Raised to 0.04 for block
	E 3.0	2.5	2.3	
8	C 8.5	5.0	5.5	Raised to 0.04 for block
	E 4.3	4.8	5.7	
10	C 6.5	3.0	1.5	NR
	E 6.5	8.0	6.2	NR

The reduction in response to C_6 1-5 μg and 10 μg in the experimental strip was not significantly different from that in the control strip at $P = 0.05$

of the experimental segment to electrical stimulation at a lower stimulus strength of 120 v at a frequency of 1 pulse/sec. It completely blocked such responses in two preparations Fig. 2.29. In three other preparations 5 $\mu\text{g/ml}$ was required to abolish the responses. Responses to 150 v and 10 pulses/sec were not reduced by hexamethonium until 1-5 $\mu\text{g/ml}$ of the drug was present. In three experiments the effects on the contractions of control strips was greater and in three experiments the effect on the contractions of the experimental strips was greater. At a hexamethonium concentration of 10 $\mu\text{g/ml}$, the magnitude of the contractions was reduced to 50% in the control and to 60-70% in the experimental strips Fig. 2.30 (A) and (B).

Atropine 0.02 $\mu\text{g/ml}$ Table 2.10: Residual responses of the jejunal strips to transmural stimulation with 150 v and 10 pulse/sec that was not blocked by hexamethonium 10 $\mu\text{g/ml}$ was tested with atropine 0.02 $\mu\text{g/ml}$. Twenty to twenty-four minutes after addition of atropine to the bath, no response to the transmural stimulation occurred in most pieces. In two preparations the concentration of atropine in the bath had to be raised to 0.04 $\mu\text{g/ml}$ before the response was completely abolished. Responses to Mch was also abolished by atropine but the muscle still responded normally to Pure KCl-Ringer solution Fig. 2.30.

Tetrodotoxin: 0.1 $\mu\text{g/ml}$. As described previously tetrodotoxin in the above doses abolished nerve action potentials by preventing the increase in Na conductance that accompanies such activity. Smooth muscle does not depend on this



Fig. 2.29. Hexamethonium stimulation of experimental jejunal strip from a chronically prepared dog. Response to low frequency stimulus (120 V and IP/S) blocked with low dose of hexamethonium (0.1 μ g - 1.0 μ g/ml).

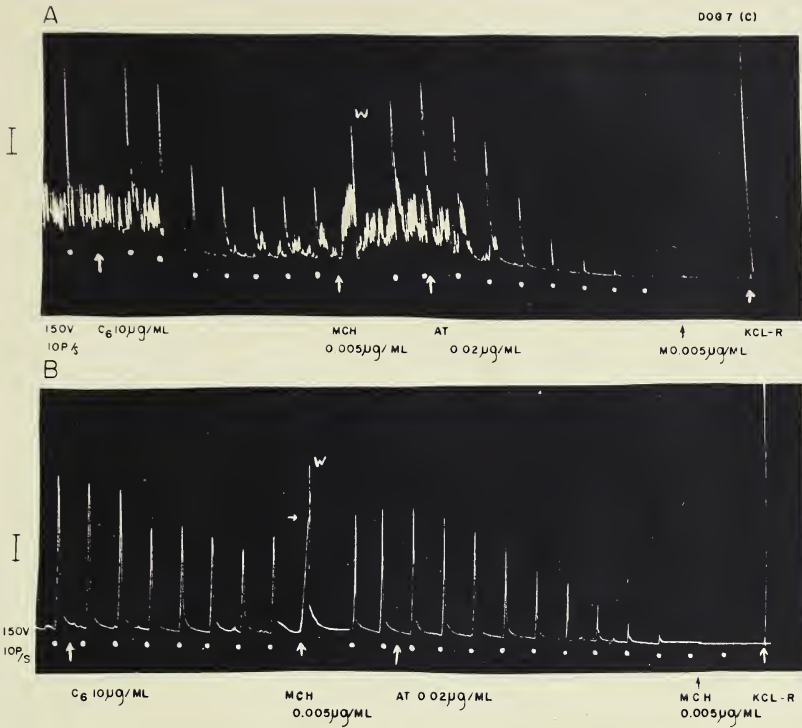


Fig. 2.30. Transmural stimulation of jejunal strips from chronically prepared dogs (A) control strip (B) experimental strip. High frequency stimulus (150V and 10 P/S.) was blocked 50% in (A) and 25% in (B) by hexamethonium (C₆) 10 µg/ml. Residual response not blocked by hexamethonium was blocked by atropine (At) 0.02 µg/ml.

TABLE 2.11

RESPONSES OF THE JEJUNUM TO REFLEX STIMULATION, DRUGS,
AND TRANSMURAL STIMULATION

C = Control, E = Experimental, (+) = Presence of a Response, NR = No Response.

Experiment No.	Response to i.a. perfusions in mm H ₂ O					
	Reflex Response	P.D.G. 10 µg	Nict. 5 µg	D.M.P.P. 2.5 µg	Mch. 0.1 µg	Transmural stimulation
6	C +	87.5	87.5	75.0	112.5	+
	E -	75.0	75.0	37.5	87.5	+
9	C +	100.0	87.5	75.0	87.5	+
	E -	75.0	75.0	25.0	75.0	+
10	C +	75.0	60.0	87.5	50.0	+
	E -	62.5	37.5	NR	50.0	+

increase in Na conductance for depolarisation and tetrodotoxin therefore has little or no effect on smooth muscle activity. As supporting evidence that the contractions in response to transmural stimulation, was due to its action on functioning nerves, the effects of tetrodotoxin on these responses were tested. Tetrodotoxin 0.1 $\mu\text{g/ml}$ completely and immediately abolished the contractile responses to transmural stimulation in all the three preparations in which this was tested. One such preparation is shown in Fig. 2.31 (A) (control) and Fig. 2.31 (B) (experimental). Tetrodotoxin in this concentration did not reduce the height of the contractions to Mch and Pure KCl Krebs Ringer solution in two preparations but did so in one. Responses to transmural stimulation reappear 30-40 minutes after tetrodotoxin had been removed from the bath and after several washings.

CONCLUSIONS

These experiments on chronically prepared dogs have presented evidence that: 1. Functioning nerves are present in intestinal segments after 4 hrs of ischaemia. 2. That after sufficient time had been allowed for muscle cells to recover, responses to stimulation of the mechanoreceptors (P.D.G.) and nerve tissue (Nict., D.M.P.P. etc) which were absent in acute preparations, could now be obtained. 3. That the absence of the reflex response does not necessarily mean complete denervation or total destruction of ganglion cells. This is shown by Table 2.11 where the

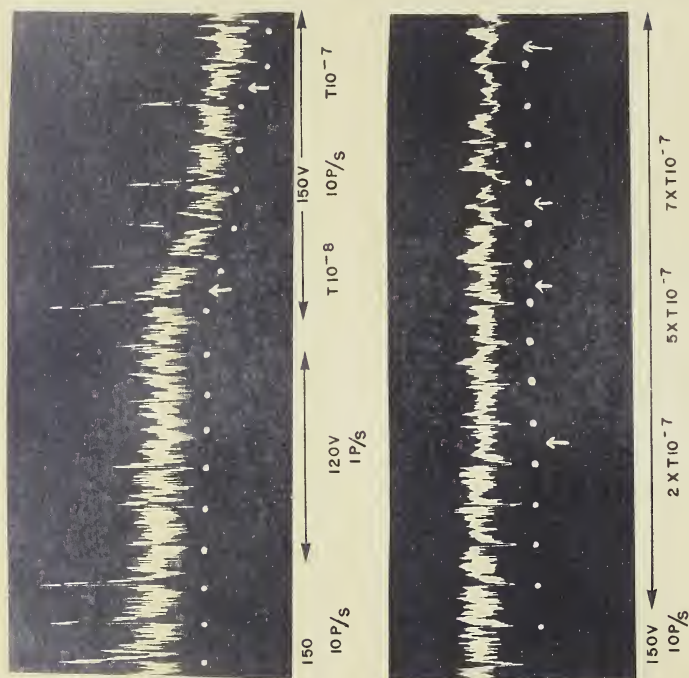


Fig. 2.31 (A) Response of a control jejunal strip from a chronically prepared dog to transmural stimulation with high frequency stimulus (150V and 10 P/S). Responses abolished by tetrodotoxin (T) 0.1 μ g/ml (10^{-7}).

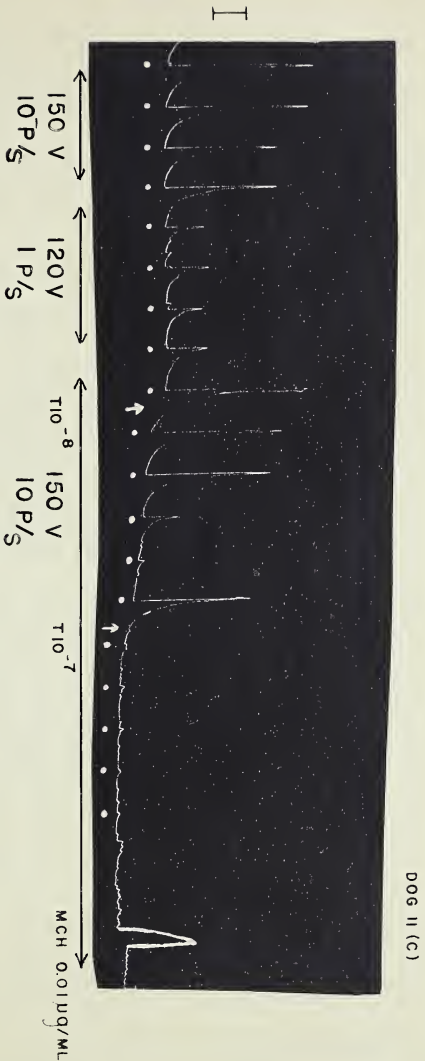


Fig. 2.31 (B) Response of an experimental jejunal strip from a chronically prepared dog to transmural stimulation with high frequency stimulation and block by tetrodotoxin (T) 0.1 $\mu\text{g}/\text{ml}$ (10^{-7}). Notice the response to Mch. 0.01 $\mu\text{g}/\text{ml}$.

three preparations in which reflex responses were seen in the control but not in the experimental preparations showed good responses to i.a. drugs which stimulate nervous elements in vivo. Transmural stimulation in such preparations also produced contractions which were reduced by hexamethonium and completely abolished by atropine. Finally in dog 10, where responses to tetrodotoxin were tested, the contraction to transmural stimulation was abolished by tetrodotoxin. Earlier, this dog in vivo showed no reflex response in the experimental segment but responded with contraction in the control segment when 0.1 N HCl was injected into the lumen Fig. 2.23.

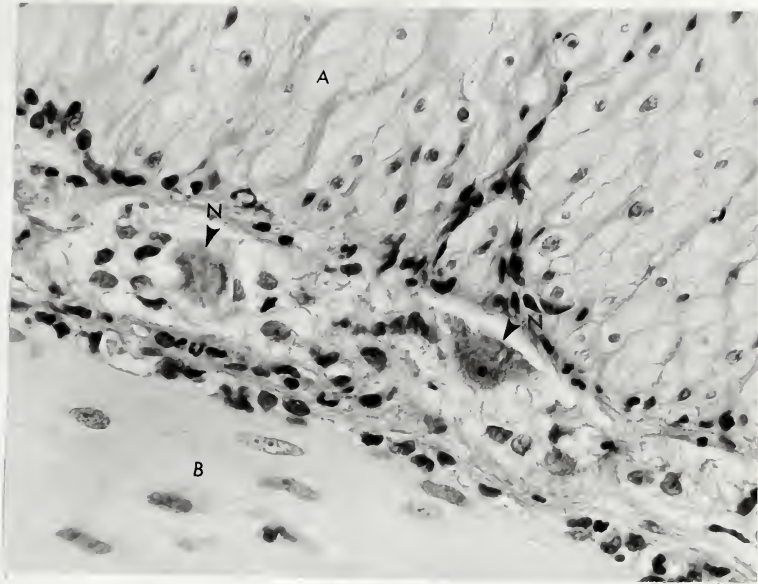
HISTOLOGICAL EXAMINATIONS (25)

At the end of in vivo experiments approximately 3 cm of jejunum was cut from the control and experimental segments and sent to the pathology laboratory for histological examination. Serial sections 5-8 μ were prepared and embedded in paraffin and stained with haematoxylin and eosin stain, Nissl's stain and thionine stain according to routine methods (179A). Results showed haemorrhagic necrosis and loss of epithelium with inflammation and oedema of muscular and neuronal elements in sections from experimental segments, all acute preparations. Some inflammatory reaction was also seen in sections from the control segments. In chronic preparations there was less of this acute inflammatory stage. Sections from the control area showed nerve elements, muscle and mucosa to be normal in appearance. The ganglion cells

Fig. 2.32 (A-D) Microscopic section of the myenteric plexus of the dog small intestine. Magnification 300 X.

- (A) From the normal control segment
- (B) From the post ischaemic segment showing ganglion cells with reversible changes
- (C) From the post ischaemic segment showing ganglion cells with irreversible changes
- (D) From the post ischaemic segment a mixture of different types,
 - A = longitudinal muscle
 - B = Circular muscle
 - R = with reversible changes
 - IR = with irreversible changes
 - N = Normal

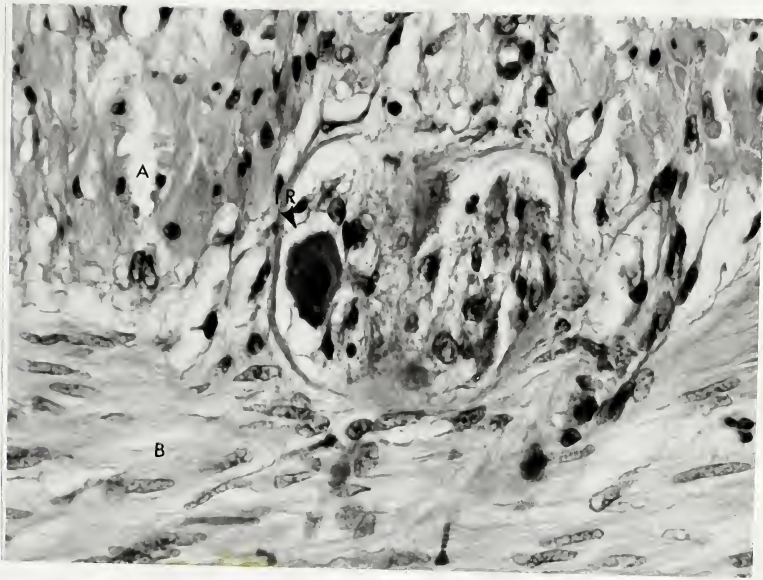
A



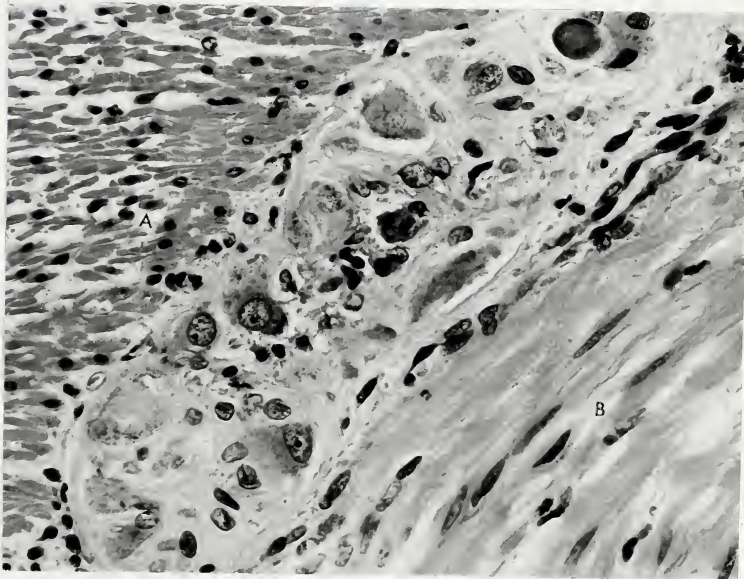
B



C



D



had a homogenous cytoplasm with a distinct nucleus, more or less in the centre of the cells. Nissl's stain showed the chromatin material evenly dispersed. In the sections from the experimental area, nerve elements showed normal ganglion cells mixed with ganglion cells undergoing various degrees of degeneration, some in the reversible stage and some irreversible. In the damaged cells there was a marked increase in cytoplasmic granularity and in many of the cells the outlines were obscured. Nissl's stain showed clumping of the chromatin material with a tendency to peripheral margination. The nucleus is darkly stained and has migrated to one pole.

TABLE 2.12

NEURON COUNTS FROM THE MYENTERIC PLEXUS OF 6 DOGS*

Method of Perfusion Series = see Methods.

Experiment No.	Method of Perfusion	Normal	Reversible Changes	Irreversible Changes
3	Simple Krebs Ringer Series	4	21	6
5	Constant Per- fusion Series	25	41	12
8	N ₂ Series	5	16	16
9	N ₂ Series	13	26	9
10	N ₂ Series	15	20	3
11	N ₂ Series	7	35	12

* All neuron counts were from single slides only.

Cells with reversible changes. Clumping of the chromatin material and a tendency to peripheral margination is present. The nucleus is dark stained but the cell outlines as well as the nucleus and nucleolus are still clearly seen. These changes when seen in pathological states are considered changes which are likely to recover for normal function.

Cells with irreversible changes. In these cells the outlines are shrunken and nucleus and nucleolus are not discernable. Vacuoles may be seen in the cell body. These cells in pathological states are considered degenerated and are not likely to recover. The number of the different types counted from slides prepared from the postischaemic segments of 6 dogs is shown in Table 2.12.

DISCUSSION

Identification of drugs which act specifically on the intrinsic nervous system, requires testing the drug on preparations in which the intramural plexus has been damaged or destroyed. Various methods for obtaining plexus-free intestinal preparations have been proposed (90, 131) but the success of such techniques in completely eliminating nerve elements is uncertain.

It has been claimed by Hukuhara et al. (34) that completely denervated and aganglionic loops of small and large intestine could be produced if isolated loops were completely emptied of their blood supply and kept ischaemic for 4 hours. In his method he used Tyrode's Ringer solution for perfusing the segments and kept the vessels clamped for 4 hours. This is similar to the procedure which has been used and described as the "stagnant method". His evidence for successful destruction of the ganglion cells was 1. A loss of intrinsic intestinal reflexes which required the participation of synapses in the myenteric plexus. He demonstrated loss of the mucosal reflex after 15 minutes of ischaemia and of the muscle reflex when the ischaemia was prolonged for 1 hour. The period required for recovery of these reflexes was proportional to the time the segment was kept ischaemic. If the period of ischaemia was more than 2.5 hours, no reflexes were seen for 6 hours which was the total duration for acute experiments. If the segment was kept ischaemic for 4 hours no recovery of the reflexes was seen even after 49 days. He therefore concluded that ischaemia of 4 hours duration completely damaged the ganglion cells of the myenteric plexus, resulting in an aganglionic loop. 2. In a recent paper (88)

he implied that after 2 weeks, the axons and nerve endings also degenerate in these preparations resulting in an aganglionic and completely denervated segment. The evidence for this implication was the absence of response to physostigmine in isolated strips prepared from aganglionic loops and the presence of contractile responses in strips from the control loops.

3. Histological evidence was the occurrence of cytolysis of ganglion cells and migration of nuclei to the periphery.

Rhythmic contractions reappeared soon after the re-establishment of blood supply. Szurszewski et al. (130), instead of the stagnant method, used constant perfusion of the segment with Tyrode's solution for 4 hours, and obtained a similar ischaemic loop. He confirmed many of the results of Hukuhara but pointed out that only 70% of the ganglion cells of Auerbach's and 10% of the ganglion cells of Meissner's were irreversibly damaged by his method. With constant perfusion he considered that 80-90% were destroyed. The criteria used for the evidence of nerve destruction was similar to that used by Hukuhara. Stimulation of the ganglia with drugs or electrically to confirm the absence of nerves, was not attempted. He studied the electrical and mechanical activity on various days after the operation, and reported the appearance of slow waves in the perfused area 7-10 days postoperative. The frequency of the slow waves in this area was lower and conduction was often from the distal control area. He concluded that slow waves may be myogenic, but that frequency was controlled by some mechanism in the myenteric plexus.

Hukuhara et al. used this "complete ischaemia" method for

producing similar conditions in the cardia of the stomach, the colon and the ileoceccolic region. It was the intention to prove or disprove pharmacologically that ischaemia of 4 hours duration did indeed destroy all nerve elements in the small intestine of the dog.

I. EVIDENCE OF FUNCTIONAL NERVES.

Results of the experiments conducted on acute as well as chronically prepared dogs indicate some degree of damage to ganglion cells. This was implied by the smaller response to D.M.P.P., Nict. and P.D.G. in the experimental segments subjected to ischaemia for 4 hours, relative to the control segment. In addition, there was complete absence or negligible reflex responses in the experimental segment compared to clear responses in the control. On the other hand they also showed a definite presence of functioning nerves. The evidence for this was 1. The response in vivo to i.a. perfusions of P.D.G., Nict., and D.M.P.P. in chronic preparations; 2. The character of the responses to transmural stimulation which was partially blocked by hexamethonium and entirely prevented by atropine. 3. Tetrodotoxin completely abolished these responses. 4. Histological evidence showed about 25% of the ganglion cells in Auerbach's plexus to be normal, about 50% had undergone reversible changes and about 25% had undergone irreversible changes.

Evidence for In Vivo Series: Smooth muscle cells as well as neurones are sensitive to anoxia but the muscle cells take less time to recover than the nerves. In acute preparations, the segment subjected to ischaemia showed no responses to i.a. perfusion of drugs, assumed to act on ganglia and nerves.

Together with this finding was the necessity of a 5-10 fold increase in the threshold dose to methacholine in these segments. Pure KCl-Ringer produced very small contractions. These results suggested that together with the destruction of nerve elements there was damage to the smooth muscle cells resulting in their reduced ability to contract. This fact was supported by the poor electrical activity recorded, irregular and distorted slow waves of low amplitude, and a complete absence of spontaneous or induced spikes. Histological findings showed intense reaction of both muscle and neuronal elements, and necrosis of the mucous membrane. In chronically prepared dogs, when sufficient time was allowed for the muscle cells to recover, contractions with P.D.G., D.M.P.P. and Nict., were readily observed in most preparations irrespective of the solution and the method of perfusion used. Spikes often accompanied the contractions. P.D.G. when injected into the aorta has been shown to stimulate non-medullated sensory receptors of the viscera and cause a release of acetylcholine at the postganglionic nerve endings (5, 10, 180). It has been used by some workers for locating the endings of the visceral afferent fibres (5, 180). In the series presented i.a. P.D.G. was used on the assumption that it stimulated the mucosal mechanoreceptors in the jejunum and gave evidence of functioning preganglionic fibres. These mechanoreceptors are insensitive to distension and only signal the presence of intestinal contents and its movements. Intra-arterial perfusion of P.D.G. in vivo, in chronically prepared dogs, produced spikes and contractions of approximately equal

magnitude in both the control and the experimental segments. The absence of response in the experimental segment of acutely prepared dog was probably due to muscle damage as a result of anoxia. Later in the series the receptors were also stimulated by application of 5-HT directly into the lumen, a reflex response being elicited as a contraction in the control segment. No such response was observed in the experimental segment. P.D.G. and 5-HT showed cross tachyphylaxis and it has been proposed that they probably act on the same receptor (180).

Nicotine and D.M.P.P. were given to test for functioning ganglion cells. Nicotine has been reported to cause contraction of intestinal muscles by stimulating intramural neurones and their axons at nicotinic cholinergic receptors (5, 133, 181). Its effects on both sites are reported to be blocked by hexamethonium, cocaine, morphine, atropine and botulinum toxin. After atropine or botulinum toxin, nicotine produced relaxation (182). This was interpreted as either an unmasked action of inhibitory neurones (possibly adrenergic) or a direct depressing action on the muscles. The direct action of nicotine on longitudinal muscle is uncertain. Botulinum toxin is reported to abolish the response of rabbit and guinea pig ileum (181) but atropine is said to have no effect (183). In larger doses nicotine depolarises the ganglion cell and produced initially a non-specific block to all ganglion stimulants, nicotine as well as the non-nicotine ones (184). Even with continued exposure, repolarisation occurs, after which a selective competitive block to drugs acting on nicotinic receptors persists.

D.M.P.P. is only quantitatively different from nicotine in the intestinal tract, but it was claimed to have less of the non-specific depolarisation action of nicotine (135, 136, 183, 185). Day and Vane (183) postulated that D.M.P.P. in equi-effective doses had more effect on cholinergic nerves and less effect on adrenergic or other inhibitory nerves, and also less effect on smooth muscles. Because of the reported greater selectivity of D.M.P.P. and of rapid restoration of response after washing, D.M.P.P. was chosen as a better test for the presence of ganglion cells in the denervated preparation. However the results were inconsistent and it has been reported (5) that D.M.P.P. also has a direct action on the longitudinal muscle cells. Therefore nicotine alone or sometimes both D.M.P.P. and nicotine were tested separately. Because of the occurrence of cross tachyphylaxis between these two drugs D.M.P.P. was given 30 minutes after nicotine. Intra-arterial perfusions produced contractions and spikes in both segments, those in the experimental area being slightly less than the control.

All these drugs exert their responses by stimulating nerve-structures in the intramural plexus. If the segment had been successively denervated and/or aganglionated then i.a. perfusions of P.D.G., D.M.P.P. and nicotine to this segment should show no response at all, or a response a great deal smaller than the control and the response to Mch should be more or less the same in both segments since the smooth muscle cells are considered to be undamaged. In acute experiments this seemed to be more or less true, but in chronically prepared intestines when the condition of the

muscle had improved the responses to drugs which stimulated nerves was present. This was taken as a suggestion that the neurones were intact and were able to function. Methacholine was chosen to demonstrate selective stimulation of the smooth muscles. Drugs which are exclusively direct acting are rare and a drug action which is shown to persist after selective destruction or blockade of nerve function is considered to be acting directly on the muscle. Methacholine produced contractions of approximately equal size on both segments or a little less in the experimental segment.

Evidence from Transmural Stimulation Series: The second evidence of the presence of functional nerves was discovered on transmural stimulation of the intestinal strips in vitro. The technique used was a modification of Paton's technique for co-axial stimulation (141). Whereas, he used the whole intestine of the guinea pig and single shocks of 50 sec duration, delivered with platinum wire electrodes, in my experiments jejunal strips were stimulated with repetitive shocks of 1-10 pulses/sec. of 5 msec duration and delivered by platinum plate electrodes.

In his original work Paton found that the twitches produced by co-axial stimulation were potentiated by eserine, not blocked by hexamethonium or by desensitization to serotonin and histamine, but were blocked by small doses of atropine. He therefore concluded that the responding structures were postganglionic cholinergic fibres. Other workers have later shown that pre- as well as postganglionic fibres could be stimulated (187, 188).

It was the intention here to stimulate the intrinsic nerve plexus and locate the site of action by the method of elimination with blocking agents known to act at certain sites. Responses to low frequency stimulus (120-150 v and 1 pulse/sec were reduced at 0.1 - 0.5 $\mu\text{g/ml}$ of hexamethonium and often completely abolished. The high frequency stimulus (120-150 v and 10 pulse/sec) was not affected till a concentration of 5-10 $\mu\text{g/ml}$ was present in the bath. The responses were then blocked 50% in the control strip and 25% in the experimental strip. This residual response was then completely abolished by atropine.

Certain assumptions have to be made in the interpretation of these results. 1. If it is assumed that after 4 hours of complete ischaemia a variable number (but not all) of ganglion cells are destroyed. 2. That there is some degree of damage to all nerve elements in the intestines, namely the pre- and postganglionic vagal fibres, the postganglionic sympathetic fibres and the afferent nerves and receptors. 3. That some nerves are affected more than others, e.g., the parasympathetic more than the sympathetic. The afferent nerves and receptors may also be the structures most affected by ischaemia.

The response obtained with low frequency stimulus was probably due to stimulation of the vagal preganglionic fibres or of the afferent part of the reflex arc for the peristaltic reflex. This response would then be sensitive to block by hexamethonium. With high frequency stimulus both the pre- and the postganglionic fibres are probably affected and possibly also the ganglion cells. If the action of the

high frequency stimulus on the postganglionic fibres was sufficient to produce a maximal or near maximal response, the effects of stimulation on the preganglionic fibres would contribute very little to the observed contractions which would therefore not be affected by hexamethonium in its ganglion blocking dose. At 10 $\mu\text{g/ml}$ hexamethonium apparently affected also the muscle cells to some extent producing the small reduction in the height of contraction in both strips. The residual response not blocked by hexamethonium was probably due to the stimulation of the postganglionic cholinergic fibres and this was abolished completely by atropine 0.02 - 0.04 $\mu\text{g/ml}$. The ability of the jejunal strips to contract in response to Mch after hexamethonium 10 $\mu\text{g/ml}$ and to Pure KCl-Ringer solution at the end of the experiment showed that the absence of a response was not due to the inability of the muscle to contract and little permanent damage to muscle had been produced by ischaemia or blocking drugs.

Tetrodotoxin has been shown to selectively interfere with the increased Na conductance that accompanies depolarisation (13, 7, 138). Tetrodotoxin abolishes the nerve action potentials which are dependent on this increase Na conductance, and has no effect on smooth muscle activity which is less dependent on this increase. The fact that tetrodotoxin completely abolished the contractile responses of both strips to transmural stimulation was another indication that the structures stimulated were nerves.

Histological Evidence: Sections taken from the post-ischaemic segments and stained with haematoxylin and eosin, and Nissl's stains showed reversible degeneration in 50% of the ganglion cells. The remaining 50% was made up of approximately equal number of normal and irreversibly damaged cells. These findings agreed with the experimental results which indicated a definite though smaller nerve response in the segment subjected to ischaemia. Therefore it would be acceptable to say that ischaemia of 4 hours duration produced 75% damage of the ganglion cells. However such a preparation cannot be called an aganglionic or a completely denervated segment, since 25% of the ganglion cells are normal and 50% are in the reversible stage. The classification of "reversible" and "irreversible" in diseased states. Under those conditions a "reversible change" indicates that the diseased nerve cell would recover eventually to perform its normal function. In the post-ischaemic preparations one cannot state with any certainty whether these reversibly damaged ganglion cells would recover sufficiently to perform normal function, or whether they are in an intermediate stage on the way to complete degeneration. One preparation studied after 21 days did not show any evidence of further degeneration but a series of experiments would be required before such a possibility can be excluded.

Reason for the Presence of Functioning Nerves.

Hukuhara et al. (34) claimed total destruction of the ganglion cells with his "complete ischaemia" method. Szurszewski et al. (130) only obtained 70% destruction in

the Auerbach's plexus and 10% in the Meissner's plexus with this technique. With constant perfusion, he claimed 80-90% destruction of the neurones. In the results presented, using both techniques, the majority of preparations showed 25% normal, 50% with varying degrees of reversible changes and 25% with irreversible changes. In vivo and in vitro experimental results also supported this finding.

Why Were All the Intestinal Intrinsic Nerves Not Destroyed?

1. Ineffective ischaemia due to faulty technique.
2. Anoxia does not destroy intrinsic nerves.
3. Hypoxia not sufficient to incapacitate the intrinsic nerves. This may be due to oxygen in the perfusing fluid or O₂ diffusing from surroundings.

I. FAULTY TECHNIQUE.

This would imply that either the isolation of the segment was incomplete, or clamping the vessels to the segment was not efficiently done. In either case, the segment would be pink from arterial blood or be cyanotic from venous blood. It has been mentioned earlier, (see Methods) that such preparations were discarded. In addition, although there was sufficient evidence to show the presence of functioning nerves, most of the observations made by other authors using this technique (34, 130) have also been observed in these experiments.

1. Loss of reflexes. This was the observation of Hukuhara (34) on which he based his assumption that ganglion cells were totally destroyed. Absence of the mucosal reflex was also

shown in the post-ischaemic segments of chronically prepared dogs in all the three preparations studied. The reflexes occurred normally in the control segment. Therefore, the loss of intrinsic reflexes does not necessarily mean complete destruction of the ganglia. It may well be that the number of ganglion cells surviving the anoxia was not sufficient to complete the reflex arc and produce a response. However, stimulation of the mechanoreceptors by P.D.G. given i.a. did elicit a contraction indicating that the afferent nerves were also not completely destroyed.

2. Absence of pendular movements. Hukuhara (88) had stated that "the ability of the cell-free strip to contract was much inferior to that of the cell-containing preparations". In the in vitro study of jejunal strips, pendular movements were not seen in most of the preparations. In two preparations slow spontaneous contractions were observed. Constant perfusion technique appears to favour these movements as they were observed in the two preparations in which this technique was used.

3. Threshold dose for contraction. Hukuhara had reported a difference in the threshold concentration required to produce a response in ganglion cell-containing and ganglion cell-free preparations. The threshold concentration of acetylcholine required to elicit a response was 10^{-10} $\mu\text{g/ml}$ in cell-containing preparations and 10^{-8} $\mu\text{g/ml}$ in cell-free preparations. As presented in "the Results" from acute in vitro experiments of strips prepared from control and post-ischaemic segments, the concentration required to produce minimal contraction was 2-5 times greater in the strips that had been subjected to ischaemia.

4. Slow waves were not observed in acutely prepared experiments but they occurred quite regularly in chronic experiments 10-14 days later. This was also the observation of Szurszewski in conducting similar experiments. The frequency of the slow wave was much lower in the experimental (8-10/min) segment than in the control (15-17/min). There was also a definite change in the shape of the slow wave. A slow positive deflection followed by a sharp fall, or a slower decline to the original potential.
5. Conduction when seen in the perfused region only occurred for short distances. Slow waves from above and below the experimental area may be conducted slowly to the electrode closest to the proximal or distal border. The asynchrony, the low frequency and the distortion of the slow waves reported by Szurszewski were also observed in these experiments. The effect of morphine on post-ischaemic segments was to increase the activity of the segment.
6. Histology. Finally histological examination of the ganglion cells showed all the features described by Hukuhara et al. (34) but a large number of normal ganglia and ganglion cells with reversible changes were also present. It has also been shown in Szurszewski's results that a large number of these cells recovered. Histological findings presented in this thesis are more in agreement with those of Szurszewski who claimed only 65-75% of the ganglion cells irreversibly destroyed.

II. THE POSSIBILITY THAT ANOXIA OF 4 HOURS DURATION DOES NOT DAMAGE THE INTRINSIC NERVES.

An assumption of this nature would require proof that a condition of anoxia does exist in the segment under study at the time or soon after the vessels were clamped. Since it would be difficult to determine oxygen content of the tissue at that time, it was not known how much oxygen there is in the segment either at the beginning or at the end of the 4 hour period. It would be interesting to measure the exact oxygen content of the muscle cells at the time with a PO_2 electrode. But such a procedure is difficult and cannot be performed at this time. The size of the nerve terminals in the myenteric plexus ranges from 0.1μ to 1.0μ in diameter. According to A.V. Hill (189) "it is extremely difficult" to produce anoxia low enough to damage nerves of this size (1 mm diameter). The critical PO_2 given was 10^{-6} atmospheres, calculated by the equation (189, pp. 217)

$$y_o = \frac{a_o r_o^2}{4k} \quad \text{where } r \text{ is the diameter of the}$$

nerve, a_o is the oxygen consumption rate, and k is the diffusion coefficient.

However one cannot assume that the intrinsic nerves kept under such low oxygen tension for 4 hours could function as efficiently as the nerves in the normal segment. Another possibility is that intestinal nerves are less dependent on the oxygen supplied externally, or that they are more dependent on glycolysis. Alternately the nerves may recover despite severe anoxia. Most of the evidence of nerve damage due to anoxia has been the evidence of impaired higher functions of the C.N.S.

It is possible that it is the functional connections that are irreversibly damaged rather than the nerve tissue itself.

III. HYPOXIA NOT SUFFICIENT TO DESTROY THE INTRINSIC NERVES COMPLETELY.

The source of oxygen in such conditions could be 1. Oxygen in the perfusion fluid. 2. Oxygen from diffusion.

Oxygen in the Perfusion Fluid. The oxygen tension of simple, non-oxygenated glucose-free Krebs Ringer solution that had been used for perfusion was tested with the Beckman oxygen sensor and was found to have a PO_2 of 12.6×10^{-2} atmospheres. Szurszewski (unpublished) had found in his experiments "a little less than the atmospheric air". Hukuhara made no mention of any particular precaution taken in any of his papers. However to reduce the oxygen content in the perfusion fluid, N_2 saturated Krebs Ringer solution was used. This solution at the end of the perfusion was tested for oxygen and was shown to have a PO_2 of 1×10^{-2} atmospheres. Oxygen consumption in a quiescent intestine is reported to be 1.17 mls/100g/min (190). Therefore in a piece of intestinal tissue weighing 10 g. (which is the approximate weight of the perfused segment) the oxygen consumption will be 11.7×10^{-2} mls/min. The intestinal H_2O reported in literature (102) is 800 cc/1000g. or 8 cc for 10 g. of the tissue. This is the approximate volume that will be replaced by the perfusion fluid at the end of the perfusion and the oxygen content of this fluid is 8×10^{-1} mls. This then is the approximate oxygen content of the fluid in the intestines at the time the vessels were clamped, i.e., at the beginning of the 4 hour period. In spite of the many approximations that have been made in the

calculations it is still apparent that with the consumption rate of 11.7×10^{-2} mls/min the oxygen in the perfusion fluid will be used up in a very short time and contribute very little to the oxygen in the tissue.

Oxygen from Diffusion: By using A.V. Hills equation (189, pp. 224) which takes in the diffusion term as well as the O_2 consumption in the steady state, P_{O_2} at a certain distance 'x' in the intestinal wall at infinite time was calculated. The equation given by Hill was:

$$y = y_0 - a_1 \frac{(1^2 - X^2)}{2k} + \frac{16 a_1 l^2}{\pi^2 3k} \left(\cos \frac{\pi X}{2 l} \right) e^{-\frac{k \pi^2 t}{4 l^2}}$$

for the 0th term

The first term $y = y_0 - a_1 \frac{(1^2 - X^2)}{2k}$ gives the P_{O_2} at a distance 'x' in the intestinal wall at the steady state i.e., at infinite time. Calculating for $x = 0, 0.25, 0.40, 0.45, 0.475$ mm it was found that at long times only the outer 0.25 mm is supplied with oxygen. The myenteric plexus is $\approx 50 \mu$ from the surface. Therefore calculating for $x = 0.990$ ($\approx 100 \mu$ from the surface) y was found to be $0.054 = 5.4 \times 10^{-2}$ atmospheres. Therefore some oxygen would always be present in the area of the myenteric plexus at all times i.e., $t = \infty$

OBSERVED EFFECTS OF ISCHAEMIA. These can be summarised as

1. Loss of pendular movements.
 2. Distortion and irregularity of slow waves.
 3. Decrease in frequency.
 4. Decrease in conduction velocity.
 5. Loss of reflex response.
- On the other hand there is no loss of the ability to respond to nerve stimulation either electrically or by drugs. Slow waves and pendular movements are assumed to be myogenic in origin and to function independently of the intrinsic or the extrinsic nerves(4,36).

Szurszewski et al. (130) postulated that the slow waves may be myogenic in origin but that their frequency and conduction are under the control of myenteric plexus which discharges impulses which increase the frequency and conduction velocity above that which is inherent to the muscle. They assumed that damage to the myenteric plexus causes the activity of the muscle to revert to the basic rate inherent to the muscle. Evidence has been presented that the myenteric plexus is not totally destroyed and that it responds to stimulation with drugs or electrically. However there could be enough damage to prevent their efficient control over slow wave frequency or their conduction.

The actual cause of failure of conduction or reduced conduction velocity in the post-ischaemic segments is not certain. That it was not the result of local damage was shown by the slow spread of activity that occurred in some segments despite the fact that ligatures had been used. Preparations from the perfusion series, show better conduction than preparations from other series. It has been suggested that functioning nerves were necessary for conduction while sufficient evidence was presented for the presence of functioning nerves in the post-ischaemic segments. Partial or complete damage to some nerves and muscle elements cannot be denied. It was difficult to assess how much of the observed results, such as the effects on slow waves and loss of reflexes, were directly due to the decreased nerve activity. It is possible that the nerves or some other structure in the intestine releases some chemical mediator e.g., acetylcholine

which is necessary for maintaining the excitability of the muscle, but if so, action of Ach not on an atropine or nicotine sensitive receptor. Ischaemia may have destroyed this ability and the absence of slow waves and pendular movements, as well as the difficulty with which spikes were induced may be the result of this destruction. If the pendular movements are in any way influenced by the pacemaker activity in the longitudinal muscle or if their presence depended upon this activity decrease in slow wave activity would be accompanied by decreased pendular movements.

Another possible cause of reduced electrical and mechanical activity was damage to the muscle elements. Interconnections between layers (54) as well as between cells (24) have been reported. Ischaemia may have damaged these interconnections and if conduction in the segment was dependent on transmission along these interconnections it was likely to be disturbed. Furthermore, damage to cell membrane, excitation-contraction coupling system or contractile system as a result of ischaemia may contribute to the lowered electrical and mechanical activity in the experimental or post-ischaemic segments.

It is apparent that the results presented do not differ basically from those presented by Hukuhara and Szurszewski (58,130). It only needs to be pointed out that loss of reflexes assumed by Hukuhara et al. to indicate absence of ganglia is not sufficient evidence for such an assumption. Segments in which no reflex response was elicited could still respond to stimulation either electrically or with drugs both of which require functioning nerves. Also the absence of response to physostigmine cannot be accepted as proof of destruction of the nerve axons.

A selective indirect action of physostigmine on nervous elements has not been established by Hukuhara et al. and it is not certain that sources of acetylcholine release, are confined to the nervous elements (28). Absence of response to physostigmine suggests but does not prove that the axons were destroyed

CONCLUSIONS.

Evidence presented in this section of the thesis indicates that ischaemia of 4 hours duration does not produce total destruction of the neurones in the intrinsic plexus. It however produced a variable degree of damage to both nerve and muscle elements and reduced their ability to respond in acute preparations.

In chronic preparations, after recovery of the tissue from acute trauma and from the reversible effects of anoxia evidence of the existence of functional nerves was obtained.

Response to transmural stimulation and the effect of hexamethonium and atropine on these responses showed that the postganglionic parasympathetic nerves were being stimulated. Effects of tetrodotoxin on these responses gave further evidence that the structures stimulated were nerves. Histological examination of stained sections from post-ischaemic segments showed a variable number of normal ganglion cells as well as cells with reversible or irreversible changes due to anoxia. It has been pointed out that absence of reflexes does not indicate that an aganglionic or a completely denervated segment was obtained.

also
It has/been pointed out that the reason for the presence of functioning nerves in these post-ischaemic segments may be due to a constant amount of oxygen available in the area of the myenteric plexus and the small oxygen requirement of these sympathetic nerves for survival or it may be due to the fact that glycolysis may have supplied the urgent need.

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